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Genome wide association study of Ethiopian barley for terminal drought stress tolerance under field and climate chamber conditions

Surafel Shibru Teklemariam[1] · Kefyalew Negisho Bayissa[2] · Andrea Matros[3] · Klaus Pillen[4] · Frank Ordon[3] · Gwendolin Wehner[3]

Abstract

In order to detect markers for drought stress tolerance, field experiments in Ethiopia were conducted for three years at two naturally drought-prone locations and two optimum moisture locations using 239 Ethiopian barley landraces and 21 barley breeding lines. Furthermore, a climate chamber experiment applying drought stress at different water regimes (70% soil water capacity (WC) for control and 20% WC for drought stress conditions) after flowering was conducted for selected 196 accessions. Results revealed reduced grain biomass by 47% and 80% under field and climate chamber conditions, respectively, as well as significantly ($p < 0.05$) reduced days to maturity and plant height, in both experimental designs. Based on 10,644 SNP markers, GWAS was conducted to identify marker trait associations (MTA) for drought stress tolerance. For days to maturity, relative chlorophyll content, plant height, number of seeds per spike, thousand kernel weight, and harvest index under field and climate chamber drought stress treatments, 58 significant MTAs were identified. In total, 41.4% of the MTAs were located on chromosome 2H, of which one is very close to the *Ppd-H1* flowering locus. These findings underpin the importance of this genome region for drought tolerance. Another MTA on chromosome 1H was detected for days to maturity under field drought stress treatment in the vicinity of the known flowering time *ELF3* gene. Additionally, 13 and 3 Ethiopian landraces that tolerate severe and moderate drought stress in climate chamber and field experiments were identified, respectively, using drought indices. The results highlight the tolerance of Ethiopian landraces to different levels of drought stress as well as their potential to be considered in future barley improvement programs.

Keywords Ethiopian barley · Landraces · Drought stress · Drought tolerance · Marker trait association

Abbreviations

MTA	Marker trait associations
GWAS	Genome wide association studies
QTLs	Quantitative trait loci
FC	Field control
FS	Field drought stress
CS	Climate chamber drought stress
CC	Climate chamber control
WC	Water capacity
DFL	Days to flowering
SPAD	Relative chlorophyll content
DM	Days to physiological maturity
PH	Plant height in cm
NSdPS	Number of seeds per spike
TKW	Thousand kernel weight in grams
GB	Grain biomass in grams
HI	Harvest index
lsmeans	Least squares means
BLINK	Bayesian-information and linkage-disequilibrium iteratively nested keyway
LD	Linkage disequilibrium

✉ Gwendolin Wehner
gwendolin.wehner@julius-kuehn.de

1 Ethiopian Institute of Agricultural Research (EIAR), Melkassa Agricultural Research Center, Melkassa, Ethiopia

2 Ethiopian Institute of Agricultural Research (EIAR), National Agricultural Biotechnology Research Center, Holetta, Ethiopia

3 Julius Kühn Institute (JKI), Federal Research Centre on Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany

4 Institute of Agricultural and Nutritional Sciences, Martin Luther University, Halle (Saale), Germany

Introduction

Crop production across Ethiopia is widely variable and highly reliant on weather conditions, which mainly vary due to the altitudinal gradient and the amount of rainfall (Gezie 2019). Almost all smallholder farmers in Ethiopia do not have supplementary irrigation facilities to ensure high yields during drought periods (Mendes and Paglietti 2015). Therefore, farmers' choice of specific crop and cultivar depends on the amount of rainfall received at that specific location (Asrat et al. 2010; Elisabeth 2004). The majority of smallholder farms (59%) in Ethiopia are in the highlands and are based on cereal farming systems with adequate rainfall, whereas farm areas in the drought-prone highlands account for 26% of the total area cultivated (Taffesse et al. 2012). Drought prone areas do not only experience low annual rainfall but also high rainfall variability (Mersha 1999).

Barley is a major staple food in the extreme highlands of Ethiopia (Shewayrga and Sopade 2011), but it is also grown in diverse agro-ecologies. It is used to prepare various food recipes (Arthur 2014; Shewayrga and Sopade 2011). Barley small-holder farmers tend to plant diversified landraces to minimize risks caused by harsh weather conditions (Di Falco and Chavas 2009). Out of the total of cereal producing farmers in Ethiopia, 24.5% are engaged in barley production, and the production covers 7.8% and 6.4% of the total cereal production area and cereal harvested yield, respectively (CSA 2019).

Ethiopia is a recognized global center of genetic diversity for barley (Harlan 1992; Vavilov 1951), and the genetic diversity is mainly influenced by the diversification of soils, climate, altitude and topography, different farming systems, together with the geographical isolation of the country for long periods (Harlan 1976; Mekonnon et al. 2015). Ethiopian barley landraces have high potential for drought tolerance and landraces collected from wide geographical locations of the country have demonstrated differences in earliness (Engels 1991), and higher yield stability with a comparative grain yield gain than improved cultivars (Abay and Bjørnstad 2009; Wosene et al. 2015). Therefore, Ethiopian landraces may use multiple drought tolerance mechanisms to alleviate the different levels of drought stress.

Genome wide association studies (GWAS) were initially used in human genetics to identify marker trait associations (MTAs) for the trait of interest (Risch and Merikangas 1996) and nowadays, it is widely used in plant genetics and breeding to identify quantitative trait loci (QTLs) for important traits (Ingvarsson and Street 2011). Many GWAS were conducted to identify genes controlling flowering time in plants, such as maize (Andersen et al. 2005; Camus-Kulandaivelu et al. 2006; Salvi et al. 2007), Arabidopsis (Olsen et al. 2004; Zhao et al. 2007), and barley (Kraakman et al. 2006), which is one of the significant traits influencing tolerance to drought stress.

GWAS were also specifically used to investigate drought tolerance in barley. Although only a few MTAs with limited phenotypic variation were reported by Varshney et al. (2012), in the succeeding studies, significant QTLs that influence the phenotype as well as the physiological and metabolic activities of juvenile barley genotypes grown under drought stress conditions were identified by Wehner et al. (2016a, b), Wehner et al. (2015), and Xiong et al. (2023). QTLs associated with seed germination (Thabet et al. 2018), water use efficiency, relative water content, and photosynthesis-related traits (Wójcik-Jagła et al. 2018), internode length and flag leaf length (Jabbari et al. 2018), spikelet organs, and number of grain per spike (Thabet et al. 2020) were identified under drought conditions. Candidate genes associated with drought tolerance that influence grain yield and associated traits (Li et al. 2022), and drought triggered environmental signals (Elbasyoni et al. 2022) were also reported. Post flowering drought experiment also revealed QTLs influencing drought tolerance in barley (Afsharyan et al. 2023).

Backcross populations developed from highly drought-tolerant Spanish landraces and elite cultivars (Monteagudo et al. 2019), and recombinant lines derived from thermo-tolerant 'Otis' cultivar and Golden Promise cultivar (Ajayi et al. 2023) were used to investigate QTLs for grain yield and related traits using the 50 k iSelect chip (Bayer et al. 2017). The result concluded that QTLs positively contributing for drought tolerance were obtained from landraces and thermo-tolerant 'Otis' cultivar.

Because Ethiopia is a center of diversity for barley and its production is in diverse eco-geographic environments, Ethiopian landraces appear to be suitable for identifying QTL for drought tolerance. The presence of molecular genetic diversity in Ethiopian barley was reported by Abebe and Léon (2013), Abebe et al. (2013), Demissie et al. (1998), and Teklemariam et al. (2022), but their drought tolerance potential was not studied using a high density marker like the 50 k iSelect chip. Thus, this study aims at identifying drought tolerant barley genotypes in Ethiopian landrace collections and their respective QTLs, and QTL regions for selected traits influencing drought stress tolerance using 50 k iSelect chip by applying GWAS.

Materials and methods

Field experiment

260 barley accessions, including 239 barley landraces provided by the Ethiopian Biodiversity Institute and 21 barley

breeding lines provided by the Holetta National Barley Improvement Program, were used for field experiments conducted for three years (2016–2018) at four locations. Field stations were at two naturally optimum moisture locations (Holetta and Debrezeit), which were designated as field control (FC) treatments and two naturally drought prone locations (Melkassa and Dera) which were assigned as field drought stress (FS) treatments. FS experiments were conducted in the central rift valley (CRV) of Ethiopia, and Kassie et al. (2013) reported that the longer dry spell in the CRV starts in September which coincides with the post flowering development cycle of barley (Figure S1a-b). Because above-average rainfall was observed in all research locations during the 2017 cropping season, no drought stress appeared, and all data from 2017 were excluded from the analyses. Furthermore, the 2018 Debrezeit data were excluded from the analyses due to the presence of poor germination at this site.

Field trials were conducted in an alpha-lattice design with three replications. Fifty seeds of each accession were sown in a single row of 1 m in length and 20 cm between rows (Figure S1a-d). The geographic coordinates, soil type, altitude, seasonal temperature, and rainfall of each research experimental location are provided in Supplementary Table S1.

Climate chamber experiment

The climate chamber experiments were conducted at the Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany with 196 accessions. These were selected by the least-square means (lsmeans) of grain biomass of 2016 drought stress locations and 2017 Holetta (control treatment) by three drought indices namely, drought susceptibility index, tolerance index, and yield reduction index, as described by Asgarinia et al. (2017). The results were used to cluster the 260 accessions into seven clusters, using SAS '*proc cluster*' procedure (SAS Institute 2019), and 75.4% of accessions were randomly selected from each cluster to accommodate a set of 196 accessions used for climate chamber experiments.

Pots with size of 15 * 15 * 20 cm were filled with 1500 g of soil (Einheitserde ED73; H. Nitsch & Sohn GmbH & Co. KG, Germany). The water capacity (WC) of the soil was determined by analyzing the weight difference between fully hydrated soil to oven dried soil according to Paech and Simonis (1952) as described in Wehner et al. (2016a, b). Three plants were grown per pot and the experiment was arranged in a split plot design with two replications. Pots were watered up to 70% WC until flowering, then the pots under drought treatment were kept at 20% WC, which was considered as climate chamber drought stress (CS) treatment and pots under optimum watering treatment were watered up

to 70% WC hereafter assigned as climate chamber control (CC) treatment (Figure S1e–h).

The climate chamber was kept at 13/11 h day/night photoperiod, with 18/14 °C day/night temperature during the vegetative growth stage and 22/16 °C day/night after flowering, and 25/18 °C day/night during the maturation period.

During the experimental period, the accessions were evaluated for several physiological parameters. Days to flowering (DFL), which was recorded at Zadoks' stages 58 (Z58) in field experiments and at Zadoks' stages 49 (Z49) in the climate chamber (Zadoks et al. 1974). Relative chlorophyll content (SPAD), which was measured using the SPAD-502 Plus instrument (Minolta, Co., Ltd., Japan) as described in Wehner et al. (2016a, b), in the field experiments, the mean measurement of three selected plants and five measurements per flag leaf a week after DFL were recorded, whereas in the climate chamber experiment, the mean measurement of three plants and five measurements per flag leaf a week after 20% WC of CS treatment were recorded. Measurements for both CC and CS treatments were taken on the same day. Days to physiological maturity (DM), which were recorded at Zadoks' stages 90 (Z90) (Zadoks et al. 1974). Plant height in cm (PH), the measurement was taken after physiological maturity (in centimeters), in field experiments, it was the mean value of five plants, while in climate chamber experiments, it was the mean value of three plants. Number of seeds per spike (NSdPS), which was the mean number of seeds per spike of ten plants in field experiments, while in the climate chamber experiment, it was the total number of harvested seeds divided by the total number of spikes. Thousand kernel weight in grams (TKW); Grain biomass in grams (GB), which represents the total harvested grains of a plot in grams. Harvest index (HI), which was the total grain biomass of ten plants in grams divided by the oven dry biomass of ten plants in grams for field experiments, while for the climate chamber experiment, the total grain biomass of three plants in grams was divided by the oven dry biomass of three plants in grams.

Statistical analyses

Statistical analysis of phenotypic data was performed with the statistics package SAS 9.4 software (SAS Institute 2019). The procedure '*proc mixed'* was used for ANOVA and estimation of least-square means (lsmeans) of traits analyzed. The model was fitted to the trait of interest as dependent variable; accessions and watering treatments were considered as fixed effects, while year, location, replication, and blocks were used as random effects. The effect of accessions interaction with treatment was also included as a fixed effect to estimate lsmeans of each trait.

The variance components and heritability (h^2) of traits were analyzed using '*lme4'* R-package (Bates et al. 2014; R

Core Team 2019), for experiments conducted for more than one year. Genotype by environment interaction and genotype by year interaction were included as random factors. Variance of genotype (σ^2_g), genotype × environment (σ^2_{gl}), genotype × year (σ^2_{gj}), residual variance components (σ^2), replication (*rep*), number of location (*nl*), and number of years (*ny*) were estimated, and broad sense heritability was calculated as:

$$h^2 = \sigma^2_g / \left(\sigma^2_g + \frac{\sigma^2_{gl}}{nl} + \frac{\sigma^2_{gj}}{ny} + \frac{\sigma^2}{nl * ny * rep} \right)$$

The Harmonic mean (HM) drought index was used in this study to identify more stable genotypes in moderate drought stress treatment (Akçura et al. 2011; Clarke et al. 1992; Guttieri et al. 2001). HM was also used to identify the most stable accessions in field trials, whereas the drought susceptible index (DSI) was used to identify drought tolerant accessions in severe drought condition like in the climate chamber experiment (Fernández 1992).

$$HM = \left[\frac{2 * (Yp * Ys)}{Yp + Ys} \right]$$

$$DSI = \left[1 - \left(\frac{Ys}{Yp} \right) \right] \Big/ \left[1 - \left(\frac{\bar{Y}s}{\bar{Y}p} \right) \right]$$

Abbreviations represent grain biomass under drought stress treatment (Ys), grain biomass under control treatment (Yp), total grain biomass mean under drought stress treatment (Ȳs), and total grain biomass mean under control treatment (Ȳp).

Genotyping

The markers from the 50 k SNP iSelect chip (SGS Trait Genetics) were filtered for < 5% missing values, > 3% minor allele frequency, and < 12.5% heterozygous SNPs resulting in a set of 10,644 SNP markers, which were used for genomic clustering in Teklemariam et al. (2022). Using these, the presence of three distinct subpopulation structure was demonstrated in Teklemariam et al. (2022) using STRUCTURE analysis (Pritchard et al. 2000), principal coordinate analysis (Perrier and Jacquemoud-Collet 2006) and neighbor-joining dendrogram (Saitou and Nei 1987). BLINK, utilizes the linkage disequilibrium information to enhance statistical power (Huang et al. 2018).

The linkage disequilibrium (LD) between all pairs of SNP markers within one chromosome was estimated using pairwise LD calculation of '*genetics*', '*Ldheatmap*' and '*trio*' R packages (R Core Team 2019; Shin et al. 2006; Warnes et al.

2013), at 0.1 critical squared allele frequency correlation (r^2) value (Oyiga et al. 2018; Voss-Fels et al. 2015).

Genome wide association studies (GWAS)

A final set of 10,644 highly polymorphic SNP markers with their physical position as well as lsmeans of phenotypic data were used to conduct GWAS. Furthermore, population structure ("PCA.total = 3") was used as correction factor. The analysis was conducted using R v.4.1.2 software (R Core Team 2021) and the "Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK)" model (Huang et al. 2018).

Markers with a false discovery rate (FDR) of adjusted *p*-value < 0.05 (Benjamini and Hochberg 1995) were considered as significant. Significant markers obtained for each trait in the workflow were analyzed to be linked based on each chromosome LD value on which they were detected, and in case the significant markers were linked, the marker with the smallest *p*-value was selected to represent the peak marker of this QTL. The identified QTLs were graphically presented using MapChart 2.32 software (Voorrips 2002). The functional annotation was analyzed by BARLEYMAP (https://floresta.eead.csic.es/barleymap) (Cantalapiedra et al. 2015) against the Morex genome v2 (Mascher et al. 2017), and genes located on the MTA positions were searched in each query and considered as candidate genes in the study.

Results

Phenotypic variation on drought stress tolerance

The presence of genetic diversity in the 260 Ethiopian genotypes was reported in Teklemariam et al. (2022). In accordance with this result, the summary of analyzed data also revealed the presence of extensive variation among the Ethiopian barley study panel for different traits (Table 1). The least coefficient of variance (CV) was observed for DM (9.4–11.9%) across all treatments and experiments. The highest CV was observed for NSdPS in FS (64.6%) and FC (64.1%), while GB (78.0%) and HI (110.8%) had the highest CV in CC and CS, respectively (Table 1).

The mean values for all traits under drought stress were smaller than the mean values under control conditions, except for DFL in the climate chamber experiment, likely because of CS and CC plants were watered at 70% WC until flowering (Figure S1e–h and Table 1). The highest reduction was observed for GB, which showed 47.4% and 79.8% reduction for FS and CS treatments compared to FC and CC treatments (Table 1). NSdPS (41.4%) and PH (32.6%) of field experiments showed a high reduction compared to other traits. Reduction of SPAD (71.2%) and

Table 1 Descriptive statistics for field experiments for the three years and for the climate chamber experiments for the two replications

Exp	Cond	Trait	Mean	Standard deviation	Minimum	Maximum	LSD ($p < 0.05$)	Coefficient of variation	Heritability	*Reduction	Number of MTAs
Field	FC	DFL	70.2	10.2	41.0	98.0	9.56	14.5	90.7%		–
		SPAD	36.08	7.39	4.30	63.30	5.94	20.48	48.7%		2
		DM	109.4	11.6	80.0	155.0	13.24	10.6	31.5%		2
		PH	108.8	15.1	62.0	154.6	17.20	13.9	50.7%		3
		GB	72.4	37.7	0.3	246.2	41.29	52.0	41.0%		6
		NSdPS	21.8	13.9	1.2	75.7	15.26	64.1	24.1%		5
		TKW	40.7	10.3	0.0	96.8	10.35	25.2	69.9%		5
		HI	0.28	0.09	0.00	1.00	0.07	31.57	12.4%		0
	FS	DFL	63.1	12.1	37.0	113.0	8.31	19.1	86.1%	10.1%	–
		SPAD	31.09	11.18	3.10	64.00	4.99	35.97	43.9%	13.8%	2
		DM	86.2	8.1	63.0	127.0	6.21	9.4	64.9%	21.3%	5
		PH	73.3	13.9	36.0	116.0	11.14	19.0	53.1%	32.6%	3
		GB	38.1	23.2	0.3	153.6	16.40	60.9	76.4%	47.4%	3
		NSdPS	12.8	8.3	1.0	51.6	6.43	64.6	48.8%	41.4%	5
		TKW	39.0	11.3	0.0	104.6	7.58	28.9	76.0%	4.2%	3
		HI	0.23	0.12	0.00	2.00	0.08	51.54	72.7%	17.9%	2
Climate chamber	CC	DFL	107.5	22.3	70.0	210.0	40.17	20.8			–
		SPAD	30.2	10.6	1.1	59.5	17.23	35.3			1
		DM	163.0	15.4	118.0	214.0	22.95	9.4			2
		PH	103.9	12.8	64.7	158.0	21.4	12.4			1
		GB	4.6	3.6	0.0	18.2	5.87	78.0			1
		NSdPS	9.6	6.0	0.3	44.9	9.2	62.3			0
		TKW	39.5	9.6	5.0	66.3	15.7	24.2			5
		HI	0.19	0.13	0.00	0.50	0.16	68.6			1
	CS	DFL	111.4	22.4	74.0	207.0	34.92	20.1		−3.%	–
		SPAD	8.7	5.0	0.8	45.8	7.60	57.0		71.2%	2
		DM	142.0	17.0	109.0	204.0	29.97	11.9		12.9%	3
		PH	97.6	11.4	53.7	140.0	17.7	11.7		6.06%	3
		GB	0.9	0.7	0.0	3.4	1.10	71.8		79.8%	4
		NSdPS	9.5	7.0	0.3	39.3	12.2	74.5		1.0%	4
		TKW	31.1	10.2	3.3	59.8	19.1	32.9		21.3%	4
		HI	0.08	0.09	0.00	0.50	0.12	110.8		57.9%	15

DFL Days to flowering, *DM* Days to maturity, *PH* Plant height in centimeter, *GB* Grain biomass in gram, *NSdPS* Number of seeds per spike, *TKW* Thousand kernel weight in gram, *HI* Harvest index, *FC* Field experiments in two naturally optimum moisture locations, *FS* Field experiments in two naturally drought prone locations, *CC* Climate chamber watering control treatment (70% WC), *CS* Climate chamber drought stress treatment (20% WC), *LSD* Least significant difference, *Reduction = ((mean of optimum − mean of deficit)/mean of optimum) *100, "–" MTA not analysed; FC and FS data of 2017 were not included as no drought stress appeared in FS locations; and 2018 Debrezeit data were excluded from FC analyses due to the presence of poor germination data at the site

HI (57.9%) of climate chamber experiments was also high. The smallest reduction was observed for TKW (4.2%) and NSdPS (1.0%) for field and climate chamber experiments, respectively (Table 1). As accessions were kept on 20% WC and in controlled environment during CS treatment, the percentage of reduction of most traits was higher in the climate chamber experiment than in the field experiments. The heritability of traits in the FS treatment ranged from $h^2 = 48.8$–86.1%; while for FC the heritability ranged from $h^2 = 12.4$–90.7% (Table 1). The heritability for GB under

FC and FS treatments was 41.0% and 76.4%, respectively, whereas the heritability of NSdPS and HI in FC was the least (Table 1).

13 accessions namely, B145.1, B191.1, B56.2, B5.1, B205, B202, B242, B213, B6, B160.2, B112.2, B143, and FTCG20 revealed DSI < 0.4 in climate chamber experiments. Three accessions namely, FTCG17, B185, and B137, were selected based on their stable GB in both FC and FS as they exhibited HM > 77.0. Among the top 50 accessions selected based on the results of DSI and HM, 7

overlapping accessions, namely B185, B191.1, FTCG2, B7, B34, FTCG13, and B260, were identified, whereas B191.1 was the only overlapping accession when the top 20 accessions from both indices were considered (Supplementary Table S2).

A highly significant genotype effect ($p < 0.001$) was observed for all traits in field and climate chamber experiments (Table 2). The interaction between accessions and treatment was also significant ($p < 0.05$) for all traits except for DM of field experiments and DFL, DM, PH, NSdPS, and TKW in climate chamber experiments. The difference between drought stress and control treatments was found to be significant ($p < 0.05$) for all traits except DFL, SPAD, TKW, and HI in field experiments, and NSdPS in climate chamber experiments (Table 2).

Table 2 ANOVA result with F-value and *P*-value for the analyzed variables in field and climate chamber experiments

Trait	Effects	Field experiment		Climate chamber experiment	
		F-value	*P*-value	F-value	*P*-value
DFL	Accessions	19.46	< 0.001	4.27	< 0.001
	Treatment	0.52	0.470	4.73	0.030
	A X T	1.76	< 0.001	0.79	0.969
SPAD	Accessions	3.07	< 0.001	2.43	< 0.001
	Treatment	0.62	0.432	1952.73	< 0.001
	A X T	1.33	< 0.001	1.41	0.002
DM	Accessions	5.63	< 0.001	3.57	< 0.001
	Treatment	3.84	0.050	502.62	< 0.001
	A X T	0.94	0.745	0.97	0.599
PH	Accessions	3.55	< 0.001	3.02	< 0.001
	Treatment	4.46	0.035	77.56	< 0.001
	A X T	1.16	0.046	0.78	0.976
GB	Accessions	3.78	< 0.001	2.03	< 0.001
	Treatment	16.76	< 0.001	490.70	< 0.001
	A X T	2.12	< 0.001	1.48	0.001
NSdPS	Accessions	7.01	< 0.001	2.78	< 0.001
	Treatment	4.39	0.036	0.73	0.394
	A X T	2.09	< 0.001	0.78	0.965
TKW	Accessions	6.96	< 0.001	1.71	< 0.001
	Treatment	0.29	0.589	150.31	< 0.001
	A X T	1.68	< 0.001	1.17	0.111
HI	Accessions	2.04	< 0.001	6.00	< 0.001
	Treatment	0.04	0.835	474.19	< 0.001
	A X T	1.37	< 0.001	1.60	< 0.001

DFL Days to flowering; *SPAD* Relative chlorophyll content; *DM* Days to maturity; *TKW* Thousand kernel weight; *NSdPS* Number of seeds per spike, *GB* Grain biomass, *PH* Average plant height; *HI* Harvest index; *A x T* Accessions and treatment interaction. Additionally, data of 2017 were not included as no drought stress appeared in field naturally drought prone locations; and 2018 Debrezeit data were excluded from FC analyses due to the presence of poor germination data at the site

Pearson's correlation coefficient (*r*) revealed a very strong positive correlation between DFL and DM of FC and FS treatment, with $r = 0.87$ and $r = 0.81$, $p < 0.001$, respectively (Table 3). In accordance, the highest correlation coefficients were observed for the climate chamber experiment between DFL and DM for CS ($r = 0.85$, $p < 0.001$) and CC ($r = 0.72$, $p < 0.001$), as well as between HI and GB ($r = 0.83$, $p < 0.001$) for CS and CC (Table 4). DFL, GB, NSdPS and HI of FS were found to be correlated with all traits (Table 3). DFL, GB and HI of CS were correlated with all traits except SPAD, and SPAD of CS was not correlated with any of the traits (Table 4).

DM was strongly negatively correlated $-0.21 \leq r \geq -0.61$, $p < 0.001$ with most traits but not correlated with PH and positively correlated $r = 0.57$, $p < 0.001$ with SPAD in FS. Similarly, DM was also strongly and negatively correlated $-0.26 \leq r \geq -0.37$, $p < 0.001$ with most traits but not correlated with TKW and SPAD in CS (Tables 2 and 3). Therefore, the correlations suggest that DM is the most important trait to identify drought tolerance in Ethiopian barley accessions rather than NSdPS, as there was a reasonable reduction (21.3% in FS and 12.9% in CS) compared to their respective control treatment and significant difference was observed due to drought stress (Tables 1 and 2).

Results obtained in climate chamber experiments of the 196 accessions were also correlated with field experiments. Positive correlations ($p < 0.001$) were detected except for SPAD of FS and CS (Table 5). The highest correlation coefficient between the drought treatments was observed for DFL ($r = 0.57$) while the smallest significant correlation coefficient was for PH ($r = 0.24$). Correlation coefficients of DM, GB and HI were ($r = 0.46$, $r = 0.40$, and HI $r = 0.47$), respectively (Table 5).

For the control treatments, HI ($r = 0.24$), NSdPS ($r = 0.38$), and DFL ($r = 0.38$) of CC were positively correlated ($p < 0.001$) with their corresponding traits estimated in FC, while PH ($r = 0.22$) at ($p < 0.01$), and DM ($r = 0.18$) and TKW ($r = 0.18$) at ($p < 0.05$) were also found to be positively correlated but GB and SPAD were not correlated, with their respective traits in CC and FC (Table 5).

Genome wide association studies (GWAS)

The overall LD decay value at r^2 of 0.1 was 3.06 Mbp. Chromosome 3H showed the largest decay of 5.08 Mbp, while chromosome 1H and 6H showed the lowest decay of 2.33 and 2.31 Mbp respectively (Table 6).

The Quantile–quantile (QQ) plots demonstrated that the majority of GWAS results were found to be efficiently fitted to the BLINK model (Figure S2). GWAS identified a total of 92 MTAs, based on false discovery rate (FDR) adjusted *p*-values < 0.05 (Benjamini and Hochberg 1995). In summary, 23, 35, 23, and 11 MTAs were identified in FS, CS,

Table 3 Pearson's correlation coefficient (*r*) analysis results for the field experiment; above the diagonal boxes for FC treatment, below the diagonal boxes for FS treatment

		Control treatment							
		DFL	SPAD	DM	PH	GB	TKW	NSdPS	HI
Drought stress treatment	DFL		0.63***	0.87***	0.10^{ns}	0.04^{ns}	0.15*	0.14*	−0.28***
	SPAD	0.60***		0.59***	0.15*	0.19**	0.15*	0.21***	$−0.06^{ns}$
	DM	0.81***	0.57***		0.16**	0.12*	0.22***	0.11^{ns}	−0.23***
	PH	−0.18**	$−0.04^{ns}$	$−0.08^{ns}$		0.43***	0.42***	0.05^{ns}	0.03^{ns}
	GB	−0.69***	−0.42***	−0.58***	0.41***		0.39***	0.15*	0.25***
	TKW	−0.35***	$−0.09^{ns}$	−0.26***	0.28***	0.29***		−0.49***	−0.15*
	NSdPS	−0.25***	−0.19**	−0.21***	0.20**	0.48***	−0.31***		0.42***
	HI	−0.70***	−0.45***	−0.61***	0.15*	0.76***	0.21***	0.45***	

DFL Days to flowering; *SPAD* Relative chlorophyll content; *DM* Days to maturity; *PH* Average plant height; *GB* Grain biomass, *TKW* Thousand kernel weight; *NSdPS* Number of seeds per spike, *HI* Harvest index; ***significant at $p < 0.001$, **significant at $p < 0.01$, *significant at $p < 0.05$, and ^{ns} for non-significant. Additionally, data of 2017 were not included as no drought stress appeared in field naturally drought prone locations; and 2018 Debrezeit data were excluded from FC analyses due to the presence of poor germination data at the site

Table 4 Pearson's correlation coefficient (*r*) analysis results for the field experiment; above the diagonal boxes for CC treatment, below the diagonal boxes for CS treatment

		Control treatment							
		DFL	SPAD	DM	PH	NSdPS	GB	TKW	HI
Drought stress treatment	DFL		$−0.08^{ns}$	0.72***	−0.28***	−0.42***	−0.55***	−0.44***	−0.70***
	SPAD	$−0.09^{ns}$		0.05^{ns}	0.16*	0.36***	0.28***	0.21**	0.20**
	DM	0.85***	$−0.02^{ns}$		−0.25***	−0.34***	−0.42***	−0.46***	−0.58***
	PH	−0.22**	0.12^{ns}	−0.26***		0.29***	0.40***	0.47***	0.34***
	NSdPS	−0.41***	0.11^{ns}	−0.36***	0.18*		0.57***	0.36***	0.67***
	GB	−0.46***	$−0.02^{ns}$	−0.37***	0.21**	0.80***		0.29***	0.83***
	TKW	−0.22***	$−0.12^{ns}$	$−0.07^{ns}$	0.20**	0.14*	0.37***		0.34***
	HI	−0.53***	$−0.03^{ns}$	−0.47***	0.14^{ns}	0.65***	0.83***	0.30***	

DFL Days to flowering; *SPAD* Relative chlorophyll content; *DM* Days to maturity; *PH* Average plant height; *GB* Grain biomass, *TKW* Thousand kernel weight; *NSdPS* Number of seeds per spike, *HI* Harvest index; ***significant at $p < 0.001$, **significant at $p < 0.01$, *significant at $p < 0.05$, and ^{ns} for non-significant

FC, and CC, respectively (Supplementary Table S3). These correspond to 84 QTLs, of which 23, 23, 27, and 11 were detected in FC, FS, CS and CC, respectively, based on the LD values of each chromosome. As 83 of 84 (98.8%) QTLs were represented by just one SNP marker (Supplementary Table S3), the main focus will be on MTAs in this study. Overall, the most MTAs were detected for HI (18), GB (14) and TKW (17); while the lowest number was detected for SPAD (7), and specifically, no markers were detected for HI in FC and NSdPS in of CC (Table 1). The highest number of 17 MTAs in drought stress treatments was detected for HI (CS = 15 and FS = 2), though 9 of the 15 CS MTAs were clustered on chromosome 2H between 711.75 and 712.33 Mbp and represented by one QTL, followed by 9 MTAs for NSdPS (FS = 5 and CS = 4) (Table 1). The least number of 4 MTAs were recorded for SPAD (FS = 2 and CS = 2) (Table 1).

Common markers detected in this study, which represent two or more MTAs were "JHI-Hv50k-2016-19711", "JHI-Hv50k-2016-31649", "JHI-Hv50k-2016-73780", "JHI-Hv50k-2016-108079", and "JHI-Hv50k-2016-281531". Apart from the marker "JHI-Hv50k-2016-31649" on chromosome 1H at 427.69 Mbp, all markers revealed at least one association under drought stress treatments (Table 7, and Supplementary Table S3). The only common marker detected between field experiments (TKW of FC) and climate chamber experiments (DM of CC; and, HI and GB of CS) was "JHI-Hv50k-2016-73780" that is located on chromosome 2H at 29.85 Mbp (Table 7 and Supplementary Table S3).

MTAs or QTLs that are at a locus of the chromosome with a physically distance less than the chromosome's LD value are considered as linked; there were 17 loci carrying 50 linked MTAs in this study (Supplementary Table S3).

Table 5 Pearson's correlation coefficient (*r*) analysis of similar traits between 196 corresponding accessions of field drought stress and climate chamber drought stress as well as for field control treatment and climate chamber control treatment

Traits	Drought stress treatments	Control treatment
	r of traits	*r* of traits
DFL	0.57***	0.38***
SPAD	0.11^{ns}	0.13^{ns}
DM	0.46***	0.17*
PH	0.24***	0.22**
NSdPS	0.52***	0.38***
TKW	0.38***	0.18*
GB	0.40***	0.11^{ns}
HI	0.47***	0.24***

DFL Days to flowering; *SPAD* Relative chlorophyll content; *DM* Days to maturity; *PH* Average plant height; *GB* Grain biomass, *TKW* Thousand kernel weight; *NSdPS* Number of seeds per spike, *HI* Harvest index; ***significant at $p < 0.001$, **significant at $p < 0.01$, *significant at $p < 0.05$, and ^{n} for non-significant

Table 6 Linkage disequilibrium (LD) decay values of Ethiopian barley landraces across the seven chromosomes based on ($r^2 = 0.1$)

Chromosome	LD decay (Mbp)
1H	2.33
2H	3.57
3H	5.08
4H	3.82
5H	2.62
6H	2.31
7H	3.82
Over all	3.06

In the drought stress treatments 32 of the 50 linked MTAs (FS = 12 and CS = 20) were detected distributed along 16 different loci, while the remaining 18 linked MTAs (FC = 10 and CC = 8) were detected in control conditions, which correspond to 13 different loci. Regarding drought stress treatments, the highest number of linked MTAs was detected for HI (15), which were distributed over six different loci that represent six QTLs followed by NSdPS, which had five linked MTAs, distributed across five different loci (Supplementary Table S3).

Few results of GWAS are in loci close to known flowering genes *Ppd-H1* and *ELF3*, which are on chromosome 2H at 29.1 Mbp and chromosome 1H at 556.9 Mb respectively, and barley row type-determining genes *Vrs1* and *Vrs2*, which are on chromosome 2H at 652.0 Mbp and chromosome 5H at 562.55 Mbp respectively (Table 7 and Supplementary Table S3). The correlation analysis showed the presence of strong associations of DFL with all traits in FS and with all

but not SPAD in CS (Tables 3 and 4), therefore the flowering determining genes may have a significant effect on different traits. The influence of six- and two-rowed barley types on the number of fertile kernels, grain yield stability, and kernel weight was described in Kandic et al. (2019), as well as the fact that the critical period to determine the grain number is much earlier than the flowering time, can be used to explain the involvement of the row type determining gene in drought stress treatments.

Linked QTLs or MTAs that are detected for the same trait in both control and drought stress treatments are considered constitutive. "JHI-Hv50k-2016-108079" was the only constitutive marker found on chromosome 2H at 654.17 Mbp for TKW in FC and FS (Table 7 and Supplementary Table S3). Furthermore, there were two constitutive MTAs detected between the same traits of climate chamber and field experiment treatments. The first one is located on chromosome 2H (674.26–676.78 Mbp) for TKW in CC and FS, while the second one is for GB in CS and FC mapped on 5H (10.35–11.77 Mbp) which also possessed constitutive markers for PH in FC and FS.

The MTAs on chromosome 2H (764.05–766.08 Mbp) were the only overlapping MTAs identified for the same trait (HI) of drought stress treatment in the field and climate chamber experiment (Table 7, Fig. 1, and Supplementary Table S3).

Both experiments' drought stress treatments revealed higher LOD values for MTAs than the control treatments. The first and the third highest LOD values were observed at the locus of multiple significant marker trait associations on chromosome 2H at 29.85 Mbp for HI (LOD = 18.6), and GB (LOD = 11.0) of CS. There were also other MTAs at this locus, namely for NSdPS (LOD = 9.0), DM (LOD = 7.2) and TKW (LOD = 5.7) of the CS, CC, and FC, respectively (Table 7). The MTA with the second highest LOD = 15.1 was found on chromosome 2H at 676.78 Mbp for TKW in FS. In LD with this MTA, there is a constitutive MTA associated with TKW in CC at 674.26 Mbp with LOD = 5.3 (Table 7, and Fig. 1). The highest LOD value for a locus carrying MTAs only in drought stress treatments was observed on 2H at 79.85 Mbp for TKW (LOD = 9.5) (Table 7). In general, the LOD values of MTAs under CS were higher than FS (Table 7).

The highest marker effect was observed for FC of GB (− 10.99), and CS of DM (− 10.35) both from chromosome 5H at 6.20, and 635.61 Mbp respectively; followed by CS of TKW (9.68) and DM (− 9.42); and FS of TKW (8.53) on chromosome 6H, 5H and 2H at 558.88, 595.35, and 676.78 Mbp, respectively; while the least effect (− 0.02 to 0.06) was observed for all significant markers observed for HI (Table 7, and Supplementary Table S3).

Six out of the seven highest PVE values were observed from the climate chamber experiment. DM of CS (33.12%),

Table 7 Summary of QTLs associated with traits under control and drought stress treatments of field and climate chamber experiment in Ethiopian barley landraces

SNP	Chr	Position (Mbp)	MAF	Effect	PVE (%)	LOD	Trt	Trait	Gene Ontologies	Annotation description
JHI-Hv50k-2016-16677	1H	27.68	0.39	−0.59	0.86	6.03	FC	DM		
JHI-Hv50k-2016-16885	1H	28.89	0.41	0.97	1.31	6.53	FS	PH	GO:0005524 GO:0006468 GO:0030247 GO:0004672 GO:0005509 GO:0005515	Receptor-like protein kinase 1
JHI-Hv50k-2016-18027	1H	36.45	0.40	0.90	1.78	7.50	FC	SPAD	GO:0005515 GO:0008270	E3 SUMO-protein ligase SIZ1
BOPA2_12_10314	1H	46.54	0.48	−1.05	1.89	5.28	FC	PH	GO:0004357 GO:0006750 GO:0042398	Glutamate–cysteine ligase B, chloroplastic
JHI-Hv50k-2016-19711	1H	72.46	0.36	−0.70	1.25	5.41	FS	SPAD	GO:0005515 GO:0008270	Ankyrin repeat-containing protein 2
JHI-Hv50k-2016-19711	1H	72.46	0.36	0.01	2.34	8.97	FS	HI	GO:0005515 GO:0008270	Ankyrin repeat-containing protein 2
JHI-Hv50k-2016-22927	1H	311.10	0.13	3.93	13.12	6.61	CS	PH	GO:0000398 GO:0005681	Thioredoxin-like protein 4A
BOPA2_12_31134	1H	357.29	0.23	−3.90	2.23	5.81	CS	TKW	GO:0015098 GO:0015689 GO:0016021	Major facilitator superfamily protein
JHI-Hv50k-2016-26918	1H	369.36	0.10	6.53	26.30	6.09	CC	DM	GO:0005515	Leucine-rich repeat family protein
JHI-Hv50k-2016-31649	1H	427.69	0.09	1.73	6.44	7.69	CC	GB		RNA recognition motif-containing protein
JHI-Hv50k-2016-31649	1H	427.69	0.09	0.06	13.65	6.49	CC	HI		RNA recognition motif-containing protein
JHI-Hv50k-2016-31649	1H	427.69	0.09	4.03	2.57	4.80	CC	TKW		RNA recognition motif-containing protein
JHI-Hv50k-2016-54103	1H	547.73	0.45	−0.84	2.33	7.57	FS	DM		N-terminal protein myristoylation
JHI-Hv50k-2016-57491	1H	556.67	0.40	4.70	4.50	6.95	CS	DM	GO:0005488 GO:0005515 GO:0005524	TATA-binding protein-associated factor 172
JHI-Hv50k-2016-58119	1H	557.95	0.10	3.20	5.64	8.17	CS	NSdPS		Structural maintenance of chromosomes 5
SCRI_RS_173604	2H	13.27	0.38	−0.02	0.00	5.13	CS	HI		Peptidase S24, S26A, S26B, S26C family protein
JHI-Hv50k-2016-67587	2H	15.46	0.04	7.67	5.42	7.46	CC	TKW		Unknown function
JHI-Hv50k-2016-72079	2H	25.15	0.27	−1.00	5.21	7.30	FC	DM	GO:0016020 GO:0055085 GO:0005215 GO:0006810 GO:0006811	Cation-chloride cotransporter 2
JHI-Hv50k-2016-73780	2H	29.85	0.15	6.74	18.66	7.20	CC	DM		Symplekin
JHI-Hv50k-2016-73780	2H	29.85	0.15	−0.34	8.32	10.95	CS	GB		Symplekin
JHI-Hv50k-2016-73780	2H	29.85	0.15	−0.06	10.01	18.59	CS	HI		Symplekin
JHI-Hv50k-2016-73780	2H	29.85	0.13	1.59	3.19	5.70	FC	TKW		Symplekin
JHI-Hv50k-2016-73929	2H	30.19	0.13	−2.84	7.08	8.96	CS	NSdPS		
SCRI_RS_110647	2H	38.25	0.18	−3.21	2.22	6.43	CC	TKW	GO:0043565 GO:0003700 GO:0006351 GO:0006355	Transcription factor-related
JHI-Hv50k-2016-77166	2H	45.57	0.12	5.40	29.40	6.59	CC	PH	GO:0003824 GO:0004553 GO:0005975	Alpha-galactosidase 2
SCRI_RS_176159	2H	48.45	0.36	−0.02	0.00	4.82	CS	HI		
SCRI_RS_152206	2H	68.79	0.38	0.92	1.72	5.52	FS	PH	GO:0019430 GO:0045454 GO:0055114 GO:0004791 GO:0005737 GO:0016491	Thioredoxin reductase
JHI-Hv50k-2016-82131	2H	79.85	0.03	5.94	18.21	9.53	FS	TKW	GO:0003779	Stomatal closure-related actin-binding protein 1

Table 7 (continued)

SNP	Chr	Position (Mbp)	MAF	Effect	PVE (%)	LOD	Trt	Trait	Gene Ontologies	Annotation description
JHI-Hv50k-2016-83709	2H	92.21	0.47	0.67	0.91	6.27	FS	NSdPS	GO:0008152 GO:0016787	Unknown protein; Has 1524 Blast hits to 1298 proteins in 225 species: Archae–9; Bacteria–84; Metazoa–474; Fungi–184; Plants–98; Viruses–17; Other Eukaryotes–658, Haloacid dehalogenase-like hydrolase (HAD) superfamily protein (source: NCBI BLink)
JHI-Hv50k-2016-87462	2H	120.87	0.03	0.41	4.30	4.78	CS	GB	GO:0005488	Exportin-T
JHI-Hv50k-2016-93122	2H	432.38	0.48	−1.26	1.22	6.82	FC	NSdPS		Unknown function
JHI-Hv50k-2016-102161	2H	620.32	0.15	−1.56	1.94	5.78	FC	TKW		Undescribed protein
JHI-Hv50k-2016-108079	2H	654.17	0.20	1.62	1.90	5.67	FC	NSdPS	GO:0003677 GO:0043565	Multiprotein bridging factor 1A
JHI-Hv50k-2016-108079	2H	654.17	0.20	−2.24	3.75	10.04	FC	TKW	GO:0003677 GO:0043565	Multiprotein bridging factor 1A
JHI-Hv50k-2016-108079	2H	654.17	0.20	−2.21	1.73	8.07	FS	TKW	GO:0003677 GO:0043565	Multiprotein bridging factor 1A
JHI-Hv50k-2016-110148	2H	674.26	0.06	4.33	5.03	5.33	CC	TKW	GO:0009081 GO:0003824 GO:0004084 GO:0008152	Branched-chain-amino-acid aminotransferase 6
SCRI_RS_115690	2H	676.78	0.04	8.53	27.28	15.07	FS	TKW		Acyl-CoA thioesterase family protein
SCRI_RS_139831	2H	702.54	0.28	−2.87	5.58	5.68	CS	PH	GO:0000105 GO:0004424	Imidazoleglycerol-phosphate dehydratase
JHI-Hv50k-2016-122549	2H	712.18	0.38	−0.02	0.00	5.17	CS	HI	GO:0016620 GO:0055114 GO:0004491 GO:0008152 GO:0016491	Aldehyde dehydrogenase family 9 member A1-B
JHI-Hv50k-2016-134459	2H	739.81	0.03	2.84	13.79	4.80	FC	TKW		Unknown function
SCRI_RS_156075	2H	764.05	0.37	−0.02	0.39	5.44	CS	HI		Translocon at the outer membrane of chloroplasts 64-V
SCRI_RS_150519	2H	766.08	0.07	−0.02	6.39	7.01	FS	HI	GO:0008270	RING, FYVE, PHD zinc finger superfamily protein
JHI-Hv50k-2016-147953	2H	767.06	0.06	1.29	3.22	4.68	FS	NSdPS	GO:0043565 GO:0003700 GO:0006355 GO:0008270	GATA transcription factor 26
JHI-Hv50k-2016-149558	3H	1.72	0.46	−3.30	1.70	6.74	CS	TKW		
SCRI_RS_189167	3H	395.20	0.24	−2.23	4.39	8.18	CS	NSdPS		Protein of unknown function (DUF1644)
JHI-Hv50k-2016-183028	3H	487.52	0.32	1.17	2.02	5.57	FC	NSdPS	alpha, beta-Hydrolases superfamily protein, undescribed protein	
JHI-Hv50k-2016-192692	3H	563.06	0.22	−1.59	2.50	5.72	CS	NSdPS		Double-stranded-RNA-binding protein 4
JHI-Hv50k-2016-228946	4H	8.82	0.11	0.30	7.88	9.63	CS	GB	GO:0005515	Transducin, WD40 repeat-like superfamily protein
JHI-Hv50k-2016-232915	4H	30.94	0.16	1.73	15.47	6.50	CS	SPAD	Cysteine proteinases superfamily protein, cathepsin B	
JHI-Hv50k-2016-252004	4H	535.42	0.38	−3.84	0.71	5.64	FC	GB		Cell cycle checkpoint protein RAD17
SCRI_RS_166159	4H	542.78	0.49	1.43	3.42	8.12	FC	PH	GO:0016020 GO:0005215 GO:0006810 GO:0006857	Protein NRT1, PTR FAMILY 8.3
JHI-Hv50k-2016-260329	4H	596.67	0.43	−1.06	2.06	9.38	FS	NSdPS		

Table 7 (continued)

SNP	Chr	Position (Mbp)	MAF	Effect	PVE (%)	LOD	Trt	Trait	Gene Ontologies	Annotation description
JHI-Hv50k-2016-260339	4H	596.91	0.46	−1.50	1.41	7.74	FC	NSdPS	GO:0043565 GO:0003677 GO:0003700 GO:0006355	Homeobox-leucine zipper protein family
JHI-Hv50k-2016-279138	5H	6.20	0.03	−10.99	7.58	6.22	FC	GB		Oxidase
JHI-Hv50k-2016-281261	5H	10.35	0.07	−0.26	3.55	5.25	CS	GB	GO:0005488	ARM repeat superfamily protein
JHI-Hv50k-2016-281531	5H	11.77	0.18	1.26	2.95	6.96	FS	PH	GO:0016021 GO:0055085	Cation, calcium exchanger 1
JHI-Hv50k-2016-281531	5H	11.77	0.18	6.39	4.44	9.77	FC	GB	GO:0016021 GO:0055085	Cation, calcium exchanger 1
JHI-Hv50k-2016-281652	5H	12.04	0.03	−2.81	10.93	5.34	FC	PH	GO:004353	Disease resistance protein (CC-NBS-LRR class) family, OJ000126_13.6 protein
SCRI_RS_180821	5H	415.99	0.21	5.03	1.61	7.31	FC	GB		
JHI-Hv50k-2016-320912	5H	562.55	0.04	0.05	9.48	6.87	CS	HI		Unknown function
JHI-Hv50k-2016-322900	5H	569.55	0.24	4.63	6.31	6.31	FC	GB	unknown function, Major facilitator super-family protein	
JHI-Hv50k-2016-323226	5H	571.20	0.30	0.80	1.98	5.41	FC	SPAD	GO:0006633 GO:0031177	Acyl carrier protein 3
JHI-Hv50k-2016-323294	5H	571.62	0.32	0.54	1.40	5.06	FS	DM	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
JHI-Hv50k-2016-325133	5H	576.05	0.03	−2.71	9.75	5.37	FC	TKW	GO:0009733	SAUR-like auxin-responsive protein family, undescribed protein
JHI-Hv50k-2016-334019	5H	595.35	0.07	−9.42	22.09	8.50	CS	DM	GO:0043565 GO:0003700 GO:0005634 GO:0006355	Heat stress transcription factor A-2a
JHI-Hv50k-2016-338412	5H	606.93	0.34	3.62	24.05	7.45	CC	SPAD		N-terminal protein myristoylation
JHI-Hv50k-2016-345216	5H	622.30	0.47	−1.25	1.55	5.94	FC	NSdPS		Unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein
JHI-Hv50k-2016-346162	5H	623.95	0.28	0.76	1.32	5.54	FS	NSdPS	GO:0004824 GO:0005524 GO:0005737 GO:0006418 GO:0006430 GO:0000166 GO:0003676 GO:0004812	Lysine–tRNA ligase
JHI-Hv50k-2016-349520	5H	635.61	0.03	−10.35	33.12	6.35	CS	DM		Triacylglycerol lipase SDP1
JHI-Hv50k-2016-388506	6H	70.92	0.14	1.06	1.54	6.25	FS	SPAD	GO:0016491 GO:0055114	Apoptosis-inducing factor homolog A
JHI-Hv50k-2016-397691	6H	344.11	0.39	−4.31	1.53	5.33	FC	GB		
JHI-Hv50k-2016-398315	6H	353.41	0.31	3.02	3.64	6.76	CC	TKW	GO:0008270	TNF receptor-associated factor 6
JHI-Hv50k-2016-398334	6H	354.34	0.18	−3.79	7.36	5.92	CS	PH	GO:0008152 GO:0016491	Dehydrogenase, reductase SDR family member 7B
JHI-Hv50k-2016-400135	6H	374.52	0.14	4.96	4.42	7.53	FS	GB	GO:0004654 GO:0006396 GO:0006402 GO:0000175 GO:0003676 GO:0003723	Polyribonucleotide nucleotidyltransferase, putative
JHI-Hv50k-2016-402539	6H	397.26	0.26	−0.77	1.35	5.53	FS	NSdPS	GO:0055114	Alcohol dehydrogenase 1
JHI-Hv50k-2016-416678	6H	538.29	0.43	−0.79	1.98	5.72	FS	DM	GO:0016491 GO:0055114	1-aminocyclopropane-1-carboxylate oxidase
JHI-Hv50k-2016-422808	6H	558.86	0.03	7.59	6.72	5.91	FS	GB	GO:0003676 GO:0005634	CCR4-NOT transcription complex subunit 7
JHI-Hv50k-2016-422853	6H	558.88	0.04	9.68	21.04	9.43	CS	TKW	GO:0005515	Pentatricopeptide repeat-containing protein
JHI-Hv50k-2016-424617	6H	563.56	0.25	−1.52	16.81	7.31	CS	SPAD	GO:0008270 GO:0016491 GO:0055114	Alcohol dehydrogenase

Table 7 (continued)

SNP	Chr	Position (Mbp)	MAF	Effect	PVE (%)	LOD	Trt	Trait	Gene Ontologies	Annotation description
SCRI_RS_621	7H	3.85	0.30	0.79	1.69	6.91	FS	DM	GO:0003677	Myb-like transcription factor family protein, undescribed protein
JHI-Hv50k-2016-450056	7H	15.06	0.08	−5.39	5.42	6.20	FS	GB	GO:0003993 GO:0005524 GO:0006468 GO:0030247 GO:0004672 GO:0005509 GO:0005515	Acid phosphatase 1, undescribed protein, Protein kinase family protein
JHI-Hv50k-2016-460705	7H	42.96	0.33	0.02	1.09	4.96	CS	HI	GO:0006355 GO:0046983 GO:0003677 GO:0003700 GO:0005634	MADS-box transcription factor 5, Retrotransposon protein, putative, unclassified
JHI-Hv50k-2016-478809	7H	272.67	0.07	−1.09	2.86	5.81	FS	DM		Disease resistance protein RPM1
BOPA2_12_30335	7H	591.55	0.47	−2.76	1.28	5.26	CS	TKW	GO:0000413 GO:0003755 GO:0006457	Peptidyl-prolyl cis–trans isomerase B

Chr Chromosome; *MAF* Minor allele frequency; *PVE(%)* Percentage of phenotype variance explained; *LOD* Logarithm of odds; *Trt* Treatment (*FS* Field drought stress; *CS* Climate chamber drought stress; *FC* Field control treatment and *CC* Climate chamber control treatment);Trait (*SPAD* Relative chlorophyll content; *DM* Days to maturity; *PH* Average plant height; *GB* Grain biomas, *TKW* Thousand kernel weight; *NSdPS* Number of seeds per spike; *HI* Harvest index); *GO term* Gene Ontologies

PH of CC (29.40%), and TKW of FS (27.28) were the three highest on chromosome 5H at 635.61 Mbp and 2H at 45.57 and 676.78 Mbp, respectively, while PVE values close to zero were recorded from HI of CS on chromosome 2H at 13.27, 48.45, and 712.18 Mbp; and from the field experiment GB (0.71%) and DM (0.86%) of FC and NSdPS of FS (0.91%), were with the least PVE values on chromosome 4H at 535.42 Mbp, 1H at 27.68 Mbp, and 2H at 92.21 Mbp, respectively (Table 7 and Supplementary Table S3).

On chromosome 2H the highest number of significant MTAs was assigned (36) with the highest LOD values, while on chromosome 3H the least number of MTAs (4) was detected (Supplementary Table S4). MTAs detected for CS were found on all chromosomes, whereas for FS on all except chromosome 3H. Furthermore, the number of MTAs detected in FS and CS on chromosomes 1H, 2H, 6H, and 7H were either equal or higher than the number of MTAs detected in FC and CC (Fig. 1). On chromosomes 2H, 6H and 1H, 16 out of 23 detected MTAs for FS are located, while on chromosomes 2H, 1H and 5H, 25 of the 35 detected MTAs in CS were detected (Supplementary Table S4).

Discussion

The amount of rainfall reduction during the main cropping season of the FS experimental locations in Ethiopia reached up to 40% compared with the FC locations (Supplementary Table S1). The FS experiments were conducted in the central rift valley (CRV) of Ethiopia, where the pattern of rainfall through the main cropping season is erratic, unpredictable, and variable compared to other locations in Ethiopia (Adimassu et al. 2014). The extended dry spell starts in general in September (Kassie et al. 2013). Bekele et al. (2019), and Bekele et al. (2016) also predicted that the pattern on the onset of the dry spell would persist in CRV at least until the end of the century.

Thus, drought stress experiments in the CRVs help in the identification of early flowering accessions that are capable of completing their life cycle before the onset of the dry period (Chaves et al. 2003). Medium-flowering accessions can also tolerate post-flowering drought stress through minimizing water loss, increasing water uptake through high root biomass, osmotic adjustment or storage of different compatible solutes (Barnabás et al. 2008; Ehleringer and Cooper 1992; Jackson et al. 2000; Yokota et al. 2006), instead of late-flowering accessions which suffer most. In the climate chamber experiments, the drought tolerance potential of all accessions was evaluated at 20% WC after flower initiation (Figure S1e-h) which enabled us to assess the potential of all accessions, including late maturing ones, unlike in the field experiment.
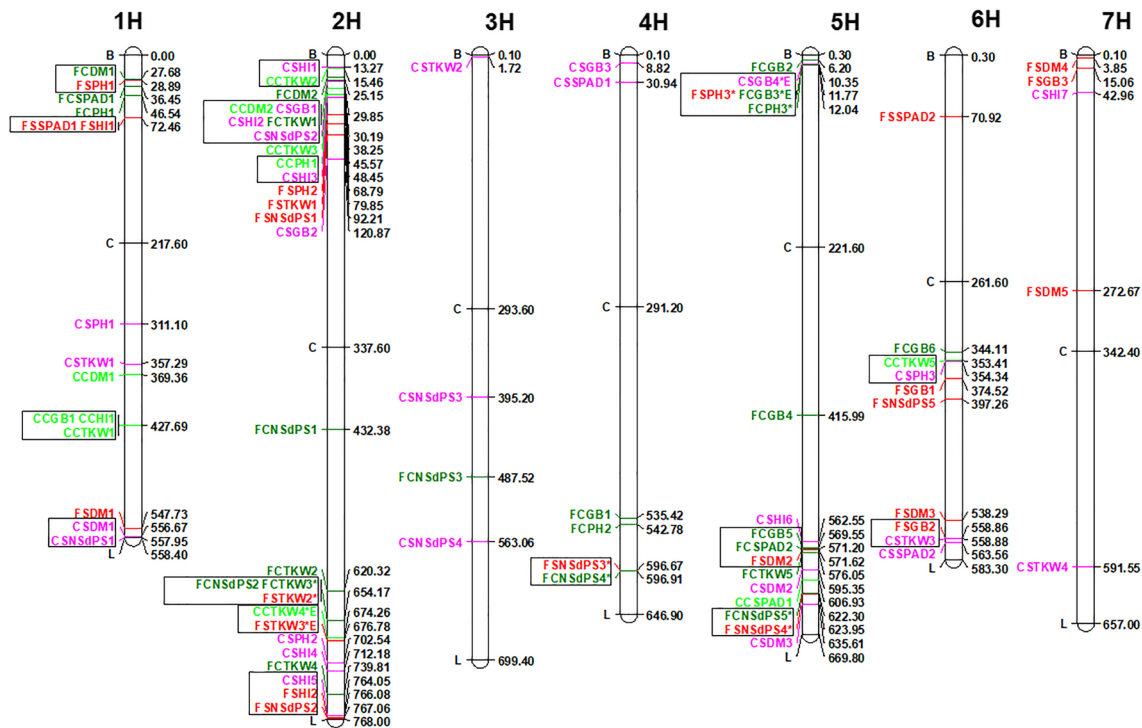
Fig. 1 Genetic map showing identified QTLs of Ethiopian barley landraces for drought tolerance traits under field and climate chamber conditions (the physical distance in Mbp). C=centromere region of the chromosome; B and L=known start and stop position of genome based on Mascher et al. (2017); QTL in dark green font=FC; red font=FS; light green font=CC; pink font=CS; QTLs inside boxes were linked; 1H to 7H for barley chromosome, the first letter "F" and "C" designated for field and climate chamber experiment, respectively; the second letter designated for treatments "C" for control treatment and "S" for drought stress treatment; the rest letter for variables "DM" for days to maturity, "GB" for grain biomass, "NSdPS" for number of seeds per spike, "PH" for plant height, "TKW" for thousand kernel weight, "HI" for harvest index; "*" constitutive QTLs, "*E" constitutive QTLs but across treatments of climate chamber and filed experiments

From Ethiopian barley landrace collections, 13 accessions were selected as tolerant to severe drought stress in the climate chamber experiment, while three landrace accessions were selected based on their stable grain biomass performance in field experiments. One accession (B191.1) was found in both experiments in the top 20 accessions (Supplementary Table S2). Nevertheless, general grain biomass reduction due to drought stress was observed, i.e. 47.4% and 79.8% in FS and CS treatments, respectively (Table 1). According to Samarah et al. (2009), drought resulted in a 73% to 87% grain yield reduction in barley, whereas Li et al. (2006) reported 25% grain yield reduction for tolerant genotypes and 50% to 55% grain yield reduction for susceptible genotypes; thus, the observed grain biomass yield reduction is consistent with previous reports.

Drought stress tolerance is considered a complex trait, especially, when the genotypes are evaluated for GB, as GB and other important agronomic traits are polygenic traits and also influenced by epistasis (Blum 2011). GB reduction by drought stress treatments was reported to be strongly correlated with different agro-physiological traits. For example, a negative correlation with DFL and DM (Vaezi et al. 2010);

positive correlation with number of spikes and NSdPS, SPAD, stomatal conductance and net photosynthesis rate (González et al. 2010; Thameur et al. 2012); negative correlation with number of tillers and biomass weight (Teulat et al. 1997, 2003) under drought stress conditions were reported. Except for SPAD, which was conversely correlated with GB in FS ($r = -0.42$, $p < 0.001$) and revealed no correlation in CS (Tables 2 and 3), our findings on drought treatments were in line with those already reported.

An acceptable rage of heritability was observed for most traits in FS treatment (Table 1). Such results are the basis to explore the influence of genetic variation on drought tolerance and enabled us to exploit the power of GWAS to detect important genomic regions associated with drought stress tolerance. Previous drought stress studies reported 80% and 64% of heritablity by Wiegmann et al. (2019), and Thabet et al. (2020) for grain yield, respectively. Our observed heritability of 76.4% for GB of FS is also in a similar range (Table 1).

Previous studies on GWAS for drought tolerance of barley detected different numbers of QTL across the barley genome. 44% of QTLs related to drought tolerance were

identified on chromosomes 2H and 3H by Zhang et al. (2017) using meta-analysis; 52% and 54% of significant markers were located on chromosomes 2H and 3H in studies by Mora et al. (2016) and Gudys et al. (2018), respectively. In other studies, the most significant markers were reported for chromosomes 2H and 4H (Wójcik-Jagła et al. 2018); and 5H (Wehner et al. 2015). In our study, on chromosome 2H 24 out of 58 (41.4%) markers associated with traits under drought stress were detected (Supplementary Table S4). Therefore, chromosome 2H was the most important for drought tolerance of Ethiopian barley landraces, not only because it had the most MTAs, but also because it had the highest LOD values (Table 7).

Genes that are expressed constantly in different environments are considered constitutive genes. Mostly, such genes are expressed at an intermediate level and provide immediate response when the environment is changing. However, to acquire an optimal level of protein from adaptive genes, the optimal environmental signal is needed (Geisel 2011). Therefore, constitutive types of genes are important to withstand mild drought stress. In this study, a pair of six constitutive QTLs distributed across five loci were detected on chromosomes 2H (2), 4H (1), and 5H (2) (Fig. 1). The intensity of drought stress in FS was moderate compared with CS. As a result, 4 out of 6 constitutive QTLs were identified in FC and FS for TKW (1), PH (1) and NSdPS (2). The remaining two constitutive QTLs were on chromosome 5H at 10.35–12.04 Mbp, for GB in FC and CS, the other for TKW in CC and FS on chromosome 2H (Fig. 1).

The constitutive QTLs for the complex trait of GB were identified in FC and CS on chromosome 5H at 10.35–11.77 Mbp (Table 7 and Fig. 1). The identification of such QTLs is important as the level of stress in CS was very high compared to FS, and another constitutive QTL was observed at this locus for PH as it was detected in FC and FS (Supplementary Table S3).

The study identified 87 significant genes, 51 of which were associated with at least one Gene Ontology (GO) term (Supplementary Table S3). GO term enrichment analysis was conducted using the Singular Enrichment Analysis tool in GO analysis toolkit and database for agricultural community, AgriGO v2.0 (https://systemsbiology.cpolar.cn/agriGOv2/index.php) (Tian et al. 2017); and, showed only one enriched GO terms based on a Bonferroni adjusted *p*-value; however, using the non-adjusted method, a total of six significant GO terms were found (Supplementary Table S5).

Three GO terms were associated with biological processes, whereas two and one were associated with molecular function, a cellular component, respectively. Specifically, the GO terms in the biological process are GO:0010468 for regulation of gene expression, GO:0060255 for regulation of macromolecule metabolic process, and GO:0019222 for regulation of metabolic process; GO terms in the molecular

function are GO:0003700 for transcription factor activity, and sequence-specific DNA binding, and GO:0003677 for DNA binding, while in the cellular component GO:0005634 was in the nucleus (Supplementary Table S5).

Except for chromosomes 3H and 7H, on which no linked QTLs were detected, other chromosomes carry at least two linked QTLs (Supplementary Table S3). One of the loci on 2H at 29.85–30.19 Mbp contains five linked QTLs (3 = CS, 1 = CC, and 1 = FC) for different traits (HI, GY, NSdPS, DM, and TKW) (Table 7 and Fig. 1); which indicates the importance of the locus to govern multiple traits. Gordon et al. (2020) also detected the association of multiple traits such as heading date, plant height, and kernel length on chromosome 2H at 27.2–29.8 Mbp in field drought stress experiments. Such effects may be due to the tight linkage of genes or the pleiotropic effect of a single gene (Hall et al. 2006). Close to these MTAs, the known barely flowering time gene (*Ppd-H1*) is located on chromosome 2H at 29.1 Mbp (Cockram et al. 2007; Mascher et al. 2017). Different studies also mentioned the importance of this locus as it carries a constitutive QTL for biomass accumulation in greenhouse experiments (Dhanagond et al. 2019), besides flowering time QTL and other grain yield related traits (Ogrodowicz et al. 2017; Thabet et al. 2018) in field and greenhouse experiments.

The locus on 2H (764.05–766.08 Mbp) is the only overlapping locus for the same trait of (HI) in CS and FS. In LD with this locus is an MTA at 767.06 Mbp for NSdPS in FS also detected (Fig. 1 and Supplementary Table S3). Overlapping MTAs between FS and CS are one of the possible indicators for the presence of similar drought tolerance mechanisms in both experiments. Although there were 17 loci, that revealed linked QTLs detected in this study, only three of them, which were mapped on chromosomes 2H (764.05–767.06 Mbp) for HI, 5H (10.35–11.77 Mbp) for GB and PH, and 6H (558.86–558.88 Mbp) for TKW and GB which also had the highest effect values, were detected in CS and FS, respectively (Fig. 1 and Supplementary Table S3). This may be due to the fact, that drought stress experiments conducted in pots do not represent actual field conditions, as pot experiments typically favor cultivars that are sensitive to ABA and give minor credit to drought tolerance mediated through high osmotic adjustment and a deep root system (Blum 2011). Furthermore, in our study at the FS locations, early flowering accessions were mostly favored over late flowering accessions, which may have efficient grain-filling capacity (Figure S1a-d).

Several studies reported different significant QTLs and candidate genes, that were close to some of the identified MTAs in our study. For example, one of the constitutive MTAs reported here for NSdPS in FS and FC was detected on chromosome 5H at 622.30–623.95 Mbp. Close to these MTAs, a QTL associated with grain yield and biomass was reported by Al-Abdallat et al. (2017) and Mora et al.

(2016), respectively in field experiments. Furthermore, on chromosome 5H at 623.5 Mbp, *HvHsfA2e*, and *Dhn9* genes, which are expressed in response to heat stress (Mikołajczak et al. 2022) and drought stress (Banerjee and Roychoudhury 2016; Graether and Boddington 2014), respectively, are located and known to be associated to biomass development during a drought stress period in Dhanagond et al. (2019). Although the reported QTL was mapped on chromosome 5H at 617.1 Mbp, an association with the number of seeds and spikelets per main spike were reported by Ogrodowicz et al. (2017).

The constitutive QTLs for TKW in field experiments were mapped on chromosome 2H at 654.17 Mbp. At 652.0 Mbp the row type determining gene *Vrs1* is located. *Vrs2* is also mapped on chromosome 5H at 564.4 Mbp. In a close proximity to the *Vrs2* locus at 562.55 Mbp on chromosome 5H, an MTA for HI in CS was detected in our study. Furthermore, a QTL associated with biomass recovery after drought stress in a greenhouse experiment was reported near to the *Vrs2* locus in Dhanagond et al. (2019), and Al-Abdallat et al. (2017) also reported a QTL associated with awn length in a drought-prone location. Six-rowed barley has more florets than two-rowed barley, allowing to produce more seeds per spike. Concordantly in our study, a strong and positive correlation of NSdPS and GB was observed (Table 3 and Table 4). Dodig et al. (2018) have shown the absence of clear differences in drought tolerance in two row type of barley but observed that six-rowed barley demonstrated a better grain yield stability while two-rowed barley showed better drought tolerance as well as better kernel weight during a defoliated drought treatment. Furthermore, Kandic et al. (2019) revealed that six-rowed barley has higher percentage of grain yield reduction than two-rowed barely in a defoliated drought stress field experiment. Therefore, the difference in row type resulting in a change in GB and TKW in our study, ultimately contributed to the difference in HI in CS treatment.

An MTA on chromosome 4H at 8.82 Mbp was also identified for GB in CS (Table 7). QTLs at LD with this locus were reported to be associated with hectoliter weight and dry biomass in drought stress treatments conducted in field and greenhouse experiments (Mora et al. 2016; Pham et al. 2019), which had a positive correlation with grain yield in these studies. In FS, one MTA with GB was mapped on chromosome 7H at 15.1 Mbp (Table 7). Although the identified QTLs were out of LD, Pham et al. (2019) reported an association with relative growth rate in a greenhouse drought experiment on barley at 10.2 Mb. The MTA on chromosome 7H, at 3.9 Mbp was found to be associated with DM in FS (Table 7). A candidate gene known as DWARF 3 (*HvD3*) was reported very close to this MTA in a greenhouse conducted experiment (Alqudah et al. 2018; Pham et al. 2019), that has a strong correlation with environmental response

and a significant association with agronomic, physiological traits and leaf blade as well as grain yield.

ELF3, also known as *eam8* was mapped on chromosome 1H at 556.9 Mb (Faure et al. 2012; Mascher et al. 2017; Zakhrabekova et al. 2012), and it promotes the transition from the vegetative development to the reproductive stage. *HvCMF6a* and *HvCMF6b*, which have similar effect like *ELF3* were also mapped on chromosome 1H located closely at 558.2 Mbp (Cockram et al. 2012; Mascher et al. 2017). In our study, MTA associated with DM in FS and CS was detected on chromosome 1H at 547.73 and 556.67 Mbp, respectively. DM had a strong correlation with DFL ($r = 0.81$, and 0.85, $p < 0.001$ for FS and CS respectively), and genes associated with DFL may have a role in DM. Additionally, an MTA for NSdPS was detected on chromosome 1H at 557.95 Mbp in CS. In field and greenhouse experiments Hu et al. (2018), and Pham et al. (2019) discovered QTL close to these loci, which have an influence on drought stress tolerance or play a role in the increase of grain yield related traits.

MTAs with the highest PVE values were on chromosome 5H and 2H at 635.61 and 676.75 Mbp for CS of DM and FS of PH with annotation of 'triacylglycerol lipase SDP1' and 'acyl-CoA thioesterase family protein', respectively (Supplementary Table S3). Perlikowski et al. (2022) explained the storage of triacylglycerol in the chloroplast during drought helps to prevent the storage of toxic fatty acids in monocots.

In rice, the overexpression of rice acyl-CoA thioesterase was reported to increase grain weight by 47% by improving the grain filling rate (Zhao et al. 2019). The presence of a significant positive association of plant height with leaf length and area of flag leaf to the fourth leaves was reported by Du et al. (2019), and in our study, the presence of a significant positive association between PH and GB in FS was observed (Table 3), which may be associated with the remobilization of important nutrients to grain during drought stress period. Additionally, maize acyl-CoA-binding proteins were reported to be expressed during drought stress, and the overexpression of the protein improves drought tolerance (Zhao et al. 2019).

Physiological maturity is an important stage at which the maximum GB and seed number are attained, and the crop stops further growth (Calderini et al. 2000). In our study, the importance of the trait was also demonstrated as it was strongly and negatively correlated ($p < 0.001$) with most other traits analysed (Table 3 and Table 4) in FS and CS. The occurrence of drought significantly reduced the time to maturity (Table 1). A number of annotations associated with DM in FS and CS were identified in this study. 1-aminocyclopropane-1-carboxylate oxidase (ACO) was one of these identified in FS (gene id of '*HORVU6Hr1G079640*') mapped on chromosome 6H at 538.29 Mbp (Supplementary Table S3). This protein was considered as a rate-limiting

enzyme in ethylene production (Houben and Van de Poel 2019), and a study demonstrated that the ratio between ABA and ethylene has a significant role in differentiating between drought tolerant and susceptible seedlings of wheat genotypes (Valluru et al. 2016).

In general, different QTLs that influenced drought stress tolerance of barley were reported by different studies (Dhanagond et al. 2019; Hu et al. 2018; Pham et al. 2019; Thabet et al. 2020, 2018), which are located in the vicinity of those detected QTLs in this study. Furthermore, QTLs were also reported around the same genome regions that influenced different traits by Abdel-Ghani et al. (2019), Al-Abdallat et al. (2017), Ogrodowicz et al. (2017), Sallam et al. (2019), and Wehner et al. (2015).

GWAS have been used to investigate agro-physiological important traits in barley (Afsharyan et al. 2023; Elbasyoni et al. 2022; Li et al. 2022; Thabet et al. 2020; Wehner et al. 2016a, b; Wehner et al. 2015; Wójcik-Jagła et al. 2018; Xiong et al. 2023), but the presence of a high false positive rate result was the major challenge (Tibbs Cortes et al. 2021). Beside the application of different *p*-values correction methods like FDR (Benjamini and Hochberg 1995) and Bonferroni methods, different GWAS models were developed to improve the computational power and efficiency. The multi-locus GWAS model of the multi-locus mixed model (MLMM) (Segura et al. 2012) had better performance than the single-locus GWAS models. Fixed and random model circulating probability unification (FarmCPU) (Liu et al. 2016) and its modified version (BLINK) had better performance than MLMM (Tibbs Cortes et al. 2021). The GWAS result obtained from this study had less false-positive result as the analysis was conducted using the BLINK model. However, to utilize the significant MTAs in the breeding program, validation of markers is required, as it ensures the marker's reliability and helps to evaluate its performance before applying them for crop improvement (Rawat 2023).

Conclusion

The drought stress experiments were conducted in naturally drought-prone areas of Ethiopia and in the climate chamber at 20% WC after flowering. The occurrence of drought reduced all the analysed traits, except DFL in the climate chamber, but the highest reduction was exhibited for GB in both experiments, which indicates that a small effect of drought in each trait can result in a high grain biomass penalty. The accession B191.1 was the only accession found to be tolerant to drought stress in the top 20 of both experiments based on HM and DSI drought indices. Strong positive correlation coefficient ($p < 0.001$) between GB and HI in CS and FS also demonstrated the potential

of the landraces in future breeding programs. The presence of an acceptable level of heritability in most traits in FS rather than in FC, and a better correlation coefficient between FS and CS than FC and CC, suggested a better adaptability of Ethiopian genotypes to different levels of drought. Using GWAS, 58 marker trait associations (23 for FS and 35 for CS) influencing drought tolerance in Ethiopian barley landraces were identified. The study found that DM was strongly associated with TKW, NSdPS, PH, SPAD, and HI traits of FS and CS treatments, indicating the importance of DM for drought tolerance. Chromosome 2H was considered most important, as it possessed the highest number (7 and 17) of MTAs for FS and CS, respectively, MTAs with the highest LOD values, as well as a locus with multiple overlapping MTAs (Fig. 1, Table 7, and Supplementary Table S3). The presence of only one overlapping MTA between a trait obtained in FS and CS, could be due to the pot experiments favouring more ABA sensitive genotypes (Blum 2011). MTAs that were close to known flowering genes such as *Ppd-H1* and *EFL3*, as well as the barley row type determining locus *Vrs1 and Vrs2* were identified. In general, this study provides an insight into the drought tolerance potential of Ethiopian barley landraces and identifies important genome regions with potential candidate genes. However, additional research will be required to validate the detected MTAs.

Author contributions The concept for the study was designed by GW and FO. The study's conception and design involved input from all authors. SST, KNB, and GW prepared the materials, collected the data, and carried out the analysis. The supervision was done by FO, KP, AM, and GW. SST conducted the data analysis and wrote the initial draft of the paper; the other authors provided feedback on earlier drafts. The final manuscript was read and approved by all authors.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Abay F, Bjørnstad A (2009) Specific adaptation of barley varieties in different locations in Ethiopia. Euphytica 167(2):181–195. https://doi.org/10.1007/s10681-008-9858-3

Abdel-Ghani AH, Sharma R, Wabila C, Dhanagond S, Owais SJ, Duwayri MA, Al-Dalain SA, Klukas C, Chen D, Lübberstedt T, von Wirén N, Graner A, Kilian B, Neumann K (2019) Genome-wide association mapping in a diverse spring barley collection reveals the presence of QTL hotspots and candidate genes for root and shoot architecture traits at seedling stage. BMC Plant Biol 19(1):216–216. https://doi.org/10.1186/s12870-019-1828-5

Abebe TD, Léon J (2013) Spatial and temporal genetic analyses of Ethiopian barley (*Hordeum vulgare* L.) landraces reveal the absence of a distinct population structure. Genet Resour Crop Evol 60(4):1547–1558. https://doi.org/10.1007/s10722-012-9941-4

Abebe TD, Mathew B, Léon J (2013) Barrier analysis detected genetic discontinuity among Ethiopian barley (*Hordeum vulgare* L.) landraces to landscape and human mobility on gene flow. Genet Resour Crop Evol 60:297–309. https://doi.org/10.1007/s10722-012-9834-6

Adimassu Z, Kessler A, Stroosnijder L (2014) Farmers' strategies to perceived trends of rainfall and crop productivity in the Central Rift Valley of Ethiopia. Environ Dev 11:123–140. https://doi.org/10.1016/j.envdev.2014.04.004

Afsharyan NP, Sannemann W, Ballvora A, Léon J (2023) Identifying developmental QTL alleles with favorable effect on grain yield components under late-terminal drought in spring barley MAGIC population. Plant Direct 7(8):e516. https://doi.org/10.1002/pld3.516

Ajayi OO, Bregitzer P, Klos K, Hu G, Walling JG, Mahalingam R (2023) QTL mapping of shoot and seed traits impacted by drought in Barley using a recombinant inbred line population. BMC Plant Biol 23(1):283. https://doi.org/10.1186/s12870-023-04292-x

Akçura M, Parfigoç F, Kaya Y (2011) Evaluating of drought stress tolerance based on selection indices in Turkish bread wheat landraces. J. Animal Plant Sci 21:700709

Al-Abdallat AM, Karadsheh A, Hadadd NI, Akash MW, Ceccarelli S, Baum M, Hasan M, Jighly A, Abu Elenein JM (2017) Assessment of genetic diversity and yield performance in Jordanian barley (*Hordeum vulgare* L.) landraces grown under Rainfed conditions. BMC Plant Biol 17(1):191. https://doi.org/10.1186/s12870-017-1140-1

Alqudah AM, Youssef HM, Graner A, Schnurbusch T (2018) Natural variation and genetic make-up of leaf blade area in spring barley. Theor Appl Genet 131(4):873–886. https://doi.org/10.1007/s00122-018-3053-2

Andersen JR, Schrag T, Melchinger AE, Zein I, Lubberstedt T (2005) Validation of Dwarf8 polymorphisms associated with flowering time in elite European inbred lines of maize (*Zea mays* L.). Theor Appl Genet 111(2):206–217. https://doi.org/10.1007/s00122-005-1996-6

Arthur JW (2014) Culinary crafts and foods in Southwestern Ethiopia: an ethnoarchaeological study of gamo groundstones and pottery. Afr Archaeol Rev 31(2):131–168

Asgarinia P, Mirlohi A, Saeidi G, Mohamadi Mirik AA, Gheysari M, Razavi VS, Charles MT (2017) Selection criteria for assessing drought tolerance in a segregating population of flax (*Linum usitatissimum* L.). Can J Plant Sci 97(3):424–437. https://doi.org/10.1139/cjps-2016-0140

Asrat S, Yesuf M, Carlsson F, Wale E (2010) Farmers' preferences for crop variety traits: lessons for on-farm conservation and technology adoption. Ecol Econ 69(12):2394–2401. https://doi.org/10.1016/j.ecolecon.2010.07.006

Banerjee A, Roychoudhury A (2016) Group II late embryogenesis abundant (LEA) proteins: structural and functional aspects in plant abiotic stress. Plant Growth Regul 79(1):1–17. https://doi.org/10.1007/s10725-015-0113-3

Barnabás B, Jäger K, Fehér A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell Environ 31(1):11–38. https://doi.org/10.1111/j.1365-3040.2007.01727.x

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823

Bayer MM, Rapazote-Flores P, Ganal M, Hedley PE, Macaulay M, Plieske J, Ramsay L, Russell J, Shaw PD, Thomas W, Waugh R (2017) Development and evaluation of a Barley 50k iSelect SNP Array. Front Plant Sci 8:1792. https://doi.org/10.3389/fpls.2017.01792

Bekele D, Alamirew T, Kebede A, Zeleke G, Melese AM (2016) Analysis of rainfall trend and variability for agricultural water management in Awash River Basin, Ethiopia. J Water Clim Change 8(1):127–141. https://doi.org/10.2166/wcc.2016.044

Bekele D, Alamirew T, Kebede A, Zeleke G, Melesse AM (2019) Modeling climate change impact on the hydrology of Keleta watershed in the Awash River Basin, Ethiopia. Environ Model Assess 24(1):95–107. https://doi.org/10.1007/s10666-018-9619-1

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate-a practical and powerful approach to multiple testing. J Roy Stat Soc B Met 57:289–300

Blum A (2011) Drought resistance–is it really a complex trait? Funct Plant Biol 38(10):753–757. https://doi.org/10.1071/FP11101

Calderini DF, Abeledo LG, Slafer GA (2000) Physiological maturity in wheat based on kernel water and dry matter. Agron J 92(5):895–901. https://doi.org/10.2134/agronj2000.925895x

Camus-Kulandaivelu L, Veyrieras JB, Madur D, Combes V, Fourmann M, Barraud S, Dubreuil P, Gouesnard B, Manicacci D, Charcosset A (2006) Maize adaptation to temperate climate: relationship between population structure and polymorphism in the Dwarf8 gene. Genetics 172(4):2449–2463. https://doi.org/10.1534/genetics.105.048603

Cantalapiedra CP, Boudiar R, Casas AM, Igartua E, Contreras-Moreira B (2015) BARLEYMAP: physical and genetic mapping of nucleotide sequences and annotation of surrounding loci in barley. Mol Breed 35(1):13. https://doi.org/10.1007/s11032-015-0253-1

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought-from genes to the whole plant. Funct Plant Biol 30(3):239–264. https://doi.org/10.1071/FP02076

Clarke JM, DePauw RM, Townley-Smith TF (1992) Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci 32(3):723–728. https://doi.org/10.2135/cropsci1992.0011183X003200030029x

Cockram J, Chiapparino E, Taylor SA, Stamati K, Donini P, Laurie DA, O'Sullivan DM (2007) Haplotype analysis of vernalization loci in European barley germplasm reveals novel VRN-H1 alleles and a predominant winter VRN-H1/VRN-H2 multi-locus

haplotype. Theor Appl Genet 115(7):993–1001. https://doi.org/10.1007/s00122-007-0626-x

Cockram J, Thiel T, Steuernagel B, Stein N, Taudien S, Bailey PC, O'Sullivan DM (2012) Genome dynamics explain the evolution of flowering time CCT domain gene families in the Poaceae. PLoS ONE 7(9):e45307. https://doi.org/10.1371/journal.pone.0045307

CSA (2019) Agricultural sample survey 2018/ 2019. Addis Ababa, Ethiopia, Central Statistical Agency. vol I

Demissie A, Bjørnstad Å, Kleinhofs A (1998) Restriction fragment length polymorphisms in landrace barleys from Ethiopia in relation to geographic, altitude, and agro-ecological factors. Crop Sci 38(1):237–243. https://doi.org/10.2135/cropsci1998.0011183X003800010040x

Dhanagond S, Liu G, Zhao Y, Chen D, Grieco M, Reif J, Kilian B, Graner A, Neumann K (2019) Non-invasive phenotyping reveals genomic regions involved in pre-anthesis drought tolerance and recovery in spring Barley. Front Plant Sci 10:1307. https://doi.org/10.3389/fpls.2019.01307

Di Falco S, Chavas J-P (2009) On crop biodiversity, risk exposure, and food security in the Highlands of Ethiopia. Am J Agr Econ 91(3):599–611. https://doi.org/10.1111/j.1467-8276.2009.01265.x

Dodig D, Kandić V, Zorić M, Nikolić-Đorić E, Nikolić A, Mutavdžić B, Perović D, Šurlan-Momirović G (2018) Comparative kernel growth and yield components of two- and six-row barley (<i>Hordeum vulgare</i>) under terminal drought simulated by defoliation. Crop Pasture Sci 69(12):1215–1224. https://doi.org/10.1071/CP18336

Du B, Liu L, Wang Q, Sun G, Ren X, Li C, Sun D (2019) Identification of QTL underlying the leaf length and area of different leaves in barley. Sci Rep 9(1):4431. https://doi.org/10.1038/s41598-019-40703-6

Ehleringer JR, Cooper TA (1992) On the role of orientation in reducing photoinhibitory damage in photosynthetic-twig desert shrubs. Plant, Cell Environ 15(3):301–306. https://doi.org/10.1111/j.1365-3040.1992.tb00977.x

Elbasyoni IS, Eltaher S, Morsy S, Mashaheet AM, Abdallah AM, Ali HG, Mariey SA, Baenziger PS, Frels K (2022) Novel single-nucleotide variants for morpho-physiological traits involved in enhancing drought stress tolerance in Barley. Plants 11(22):3072

Elisabeth M-H (2004) Contrasting climate variability and meteorological drought with perceived drought and climate change in northern Ethiopia. Clim Res 27(1):19–31

Engels JMM (1991) A diversity study in Ethiopian barley. In: Hawkes JG, Engels JMM, Worede M (eds) Plant genetic resources of Ethiopia. Cambridge University Press, Cambridge, pp 131–139. https://doi.org/10.1017/CBO9780511551543.010

Faure S, Turner AS, Gruszka D, Christodoulou V, Davis SJ, Korff Laurie MVDA (2012) Mutation at the circadian clock gene <i>EARLY MATURITY 8</i> adapts domesticated barley (<i>Hordeum vulgare</i>) to short growing seasons. Proc Natl Acad Sci 109(21):8328–8333. https://doi.org/10.1073/pnas.1120496109

Fernández GCJ (1992) Effective selection criteria for assessing plant stress tolerance. In: Proceedings of the international symposium on "adaptation of vegetables and other food crops in temperature and water stress. Taiwan, AVRDC Publication

Geisel N (2011) Constitutive versus responsive gene expression strategies for growth in changing environments. PLoS ONE 6(11):e27033–e27033. https://doi.org/10.1371/journal.pone.0027033

Gezie M (2019) Farmer's response to climate change and variability in Ethiopia: a review. Cogent Food Agric 5(1):1613770. https://doi.org/10.1080/23311932.2019.1613770

González A, Bermejo V, Gimeno BS (2010) Effect of different physiological traits on grain yield in barley grown under irrigated and terminal water deficit conditions. J Agric Sci 148(03):319–328. https://doi.org/10.1017/S0021859610000031

Gordon T, Wang R, Bowman B, Klassen N, Wheeler J, Bonman JM, Bockelman H, Chen J (2020) Agronomic and genetic assessment of terminal drought tolerance in two-row spring barley. Crop Sci 60(3):1415–1427. https://doi.org/10.1002/csc2.20040

Graether SP, Boddington KF (2014) Disorder and function: a review of the dehydrin protein family. Front Plant Sci 5:576. https://doi.org/10.3389/fpls.2014.00576

Gudys K, Guzy-Wrobelska J, Janiak A, Dziurka MA, Ostrowska A, Hura K, Jurczyk B, Żmuda KA, Grzybkowska D, Śróbka J, Urban W, Biesaga-Koscielniak J, Filek M, Koscielniak J, Mikołajczak K, Ogrodowicz P, Krystkowiak K, Kuczyńska A, Krajewski P, Szarejko I (2018) Prioritization of candidate genes in QTL regions for physiological and biochemical traits underlying drought response in barley (<i>Hordeum vulgare</i> L.). Front Plant Sci 9:769. https://doi.org/10.3389/fpls.2018.00769

Guttieri MJ, Stark JC, O'Brien K, Souza E (2001) Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. Crop Sci 41(2):327–335. https://doi.org/10.2135/cropsci2001.412327x

Hall MC, Basten CJ, Willis JH (2006) Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life-history variation in <i>Mimulus guttatus</i>. Genetics 172(3):1829–1844. https://doi.org/10.1534/genetics.105.051227

Harlan J (1976) Evolution of crop plants. In: Simmonds N (ed) Barley. Longman Press, UK, pp 93–98

Harlan JR (1992) Crops & man. In: Madison WI (ed) American society of agronomy. Crop Science Society of America, USA

Houben M, Van de Poel B (2019) 1-aminocyclopropane-1-carboxylic acid oxidase (ACO): the enzyme that makes the plant hormone ethylene. Front Plant Sci 10:695. https://doi.org/10.3389/fpls.2019.00695

Hu X, Zuo J, Wang J, Liu L, Sun G, Li C, Ren X, Sun D (2018) Multi-locus genome-wide association studies for 14 main agronomic traits in barley. Front Plant Sci 9:1683. https://doi.org/10.3389/fpls.2018.01683

Huang M, Liu X, Zhou Y, Summers RM, Zhang Z (2018) BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. GigaScience 8(2):giy154. https://doi.org/10.1093/gigascience/giy154

Ingvarsson PK, Street NR (2011) Association genetics of complex traits in plants. New Phytol 189(4):909–922. https://doi.org/10.1111/j.1469-8137.2010.03593.x

SAS Institute (2019). Base SAS® 9.4 procedures guide, (7th edn)

Jabbari M, Fakheri BA, Aghnoum R, Mahdi Nezhad N, Ataei R (2018) GWAS analysis in spring barley (<i>Hordeum vulgare</i> L.) for morphological traits exposed to drought. PLoS ONE 13(9):e0204952. https://doi.org/10.1371/journal.pone.0204952

Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport: using physiological processes in global predictions. Trends Plant Sci 5(11):482–488. https://doi.org/10.1016/S1360-1385(00)01766-0

Kandic V, Dodig D, Secanski M, Prodanovic S, Brankovic G, Titan P (2019) Grain yield, agronomic traits, and protein content of two-and six-row barley genotypes under terminal drought conditions. Chil J Agric Res 79:648–657. https://doi.org/10.4067/S0718-58392019000400648

Kassie BT, Rötter RP, Hengsdijk H, Asseng S, Van Ittersum MK, Kahiluoto H, Van Keulen H (2014) Climate variability and change in the central Rift Valley of Ethiopia: challenges for rainfed crop production. J Agric Sci 152(1):58–74. https://doi.org/10.1017/S0021859612000986

Kraakman ATW, Martínez F, Mussiraliev B, Eeuwijk FA, Niks RE (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Mol Breed 17(1):41–58. https://doi.org/10.1007/s11032-005-1119-8

Li R-H, Guo P-G, Michael B, Stefania G, Salvatore C (2006) Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agric Sci China 5(10):751–757. https://doi.org/10.1016/S1671-2927(06)60120-X

Li J, Yao X, Yao Y, An L, Feng Z, Wu K (2022) Genome-wide association mapping of hulless barely phenotypes in drought environment. Front Plant Sci 13:924892. https://doi.org/10.3389/fpls.2022.924892

Liu X, Huang M, Fan B, Buckler ES, Zhang Z (2016) Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet 12(2):e1005767–e1005767. https://doi.org/10.1371/journal.pgen.1005767

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J, Bayer M, Ramsay L, Liu H, Haberer G, Zhang X-Q, Zhang Q, Barrero RA, Li L, Taudien S, Groth M, Felder M, Hastie A, Šimková H, Staňková H, Vrána J, Chan S, Muñoz-Amatriaín M, Ounit R, Wanamaker S, Bolser D, Colmsee C, Schmutzer T, Aliyeva-Schnorr L, Grasso S, Tanskanen J, Chailyan A, Sampath D, Heavens D, Clissold L, Cao S, Chapman B, Dai F, Han Y, Li H, Li X, Lin C, McCooke JK, Tan C, Wang P, Wang S, Yin S, Zhou G, Poland JA, Bellgard MI, Borisjuk L, Houben A, Doležel J, Ayling S, Lonardi S, Kersey P, Langridge P, Muehlbauer GJ, Clark MD, Caccamo M, Schulman AH, Mayer KFX, Platzer M, Close TJ, Scholz U, Hansson M, Zhang G, Braumann I, Spannagl M, Li C, Waugh R, Stein N (2017) A chromosome conformation capture ordered sequence of the barley genome. Nature 544:427. https://doi.org/10.1038/nature22043

Mekonnon B, Lakew B, Dessalegn T (2015) Morphological diversity and association of traits in ethiopian food barley (*Hordeum vulgare* l.) landraces in relation to regions of origin and altitudes. J Plant Breed Crop Sci 7:44–54. https://doi.org/10.5897/JPBCS2014.0480

Mendes DM, Paglietti L (2015) Ethiopia irrigation market brief, country highlights. FAO/IFC cooperation, food and agriculture organization of The United Nations, Rome

Mersha E (1999) Annual rainfall and potential evapotranspiration in Ethiopia. Ethiop J Nat Resour 1:137–154

Mikołajczak K, Kuczyńska A, Ogrodowicz P, Kiełbowicz-Matuk A, Ćwiek-Kupczyńska H, Daszkowska-Golec A, Szarejko I, Surma M, Krajewski P (2022) High-throughput sequencing data revealed genotype-specific changes evoked by heat stress in crown tissue of barley sdw1 near-isogenic lines. BMC Genom 23(1):177–177. https://doi.org/10.1186/s12864-022-08410-1

Monteagudo A, Casas AM, Cantalapiedra CP, Contreras-Moreira B, Gracia MP, Igartua E (2019) Harnessing novel diversity from landraces to improve an elite barley variety. Front Plant Sci 10:434. https://doi.org/10.3389/fpls.2019.00434

Mora F, Quitral YA, Matus I, Russell J, Waugh R, del Pozo A (2016) SNP-based QTL mapping of 15 complex traits in barley under rain-fed and well-watered conditions by a mixed modeling approach. Front Plant Sci 7:909. https://doi.org/10.3389/fpls.2016.00909

Ogrodowicz P, Adamski T, Mikołajczak K, Kuczyńska A, Surma M, Krajewski P, Sawikowska A, Górny AG, Gudyś K, Szarejko I, Guzy-Wróbelska J, Krystkowiak K (2017) QTLs for earliness and yield-forming traits in the Lubuski × CamB barley RIL population under various water regimes. J Appl Genet 58(1):49–65. https://doi.org/10.1007/s13353-016-0363-4

Olsen KM, Halldorsdottir SS, Stinchcombe JR, Weinig C, Schmitt J, Purugganan MD (2004) Linkage disequilibrium mapping of Arabidopsis CRY2 flowering time alleles. Genetics 167(3):1361–1369. https://doi.org/10.1534/genetics.103.024950

Oyiga BC, Sharma RC, Baum M, Ogbonnaya FC, Léon J, Ballvora A (2018) Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. Plant, Cell Environ 41(5):919–935. https://doi.org/10.1111/pce.12898

Paech K, Simonis W (1952) Pflanzenphysiologische Praktika Band I Übungen zur Stoffwechselphysiologie der Pflanzen. Berlin Göttingen Heidelberg, Springer Verlag, Berlin

Perlikowski D, Lechowicz K, Skirycz A, Michaelis Ä, Pawłowicz I, Kosmala A (2022) The role of triacylglycerol in the protection of cells against lipotoxicity under drought in *Lolium multiflorum/Festucaarundinacea* introgression forms. Plant Cell Physiol 63(3):353–368. https://doi.org/10.1093/pcp/pcac003

Perrier X, Jacquemoud-Collet J (2006) DARwin software. Dissimilarity analysis and represntation for Windows

Pham A-T, Maurer A, Pillen K, Brien C, Dowling K, Berger B, Eglinton JK, March TJ (2019) Genome-wide association of barley plant growth under drought stress using a nested association mapping population. BMC Plant Biol 19(1):134. https://doi.org/10.1186/s12870-019-1723-0

Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

R Core Team (2019) R version 3.6.1. A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. Available online at: http://www.R-project.org/

R Core Team (2021) R version 4.2.1. A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. Available online at: http://www.R-project.org/

Rawat S (2023) Emerging frontiers in plant breeding molecular markers and nanopriming for enhanced crop improvement. Nanopriming Approach Sustain Agric 159–174

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273(5281):1516–1517. https://doi.org/10.1126/science.273.5281.1516

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sallam A, Alqudah AM, Dawood MFA, Baenziger PS, Börner A (2019) Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. Int J Mol Sci 20(13):3137. https://doi.org/10.3390/ijms20133137

Salvi S, Sponza G, Morgante M, Tomes D, Niu X, Fengler KA, Meeley R, Ananiev EV, Svitashev S, Bruggemann E, Li B, Hainey CF, Radovic S, Zaina G, Rafalski JA, Tingey SV, Miao GH, Phillips RL, Tuberosa R (2007) Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize. Proc Natl Acad Sci U S A 104(27):11376–11381. https://doi.org/10.1073/pnas.0704145104

Samarah NH, Alqudah AM, Amayreh JA, McAndrews GM (2009) The effect of late-terminal drought stress on yield components of four barley cultivars. J Agron Crop Sci 195(6):427–441. https://doi.org/10.1111/j.1439-037X.2009.00387.x

Segura V, Vilhjálmsson BJ, Platt A, Korte A, Seren Ü, Long Q, Nordborg M (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat Genet 44(7):825–830

Shewayrga H, Sopade PA (2011) Ethnobotany, diverse food uses, claimed health benefits and implications on conservation of barley landraces in North Eastern Ethiopia highlands. J Ethnobiol Ethnomed 7(1):19. https://doi.org/10.1186/1746-4269-7-19

Shin J-H, Blay S, McNeney B, Graham J (2006) LDheatmap: an R function for graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. J Stat Softw 16:1–9

Taffesse AS, Dorosh P, Asrat S (2012) Crop production in Ethiopia: regional patterns and trends Ethiopia strategy support program (ESSP II), International food policy research institute, and Ethiopian development research institute. Research Note 11:2

Teklemariam SS, Bayissa KN, Matros A, Pillen K, Ordon F, Wehner G (2022) The genetic diversity of Ethiopian barley genotypes in relation to their geographical origin. PLoS ONE 17(5):e0260422. https://doi.org/10.1371/journal.pone.0260422

Teulat B, Rekika D, Nachit MM, Monneveux P (1997) Comparative osmotic adjustments in barley and tetraploid wheats. Plant Breed 116(6):519–523. https://doi.org/10.1111/j.1439-0523.1997.tb02183.x

Teulat B, Zoumarou-Wallis N, Rotter B, Ben Salem M, Bahri H, This D (2003) QTL for relative water content in field-grown barley and their stability across mediterranean environments. Theor Appl Genet 108(1):181–188. https://doi.org/10.1007/s00122-003-1417-7

Thabet SG, Moursi YS, Karam MA, Graner A, Alqudah AM (2018) Genetic basis of drought tolerance during seed germination in barley. PLoS ONE 13(11):e0206682. https://doi.org/10.1371/journal.pone.0206682

Thabet SG, Moursi YS, Karam MA, Börner A, Alqudah AM (2020) Natural variation uncovers candidate genes for barley spikelet number and grain yield under drought stress. Genes 11(5):533

Thameur A, Lachiheb B, Ferchichi A (2012) Drought effect on growth, gas exchange and yield, in two strains of local barley Ardhaoui, under water deficit conditions in southern Tunisia. J Environ Manag 113:495–500. https://doi.org/10.1016/j.jenvman.2012.05.026

Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z (2017) agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Res 45(W1):W122–W129. https://doi.org/10.1093/nar/gkx382

Tibbs Cortes L, Zhang Z, Yu J (2021) Status and prospects of genome-wide association studies in plants. Plant Genom 14(1):e20077. https://doi.org/10.1002/tpg2.20077

Vaezi B, Bavei V, Shiran B (2010) Screening of barley genotypes for drought tolerance by agro-physiological traits in field condition. Afr J Agric Res 5(9):881–892

Valluru R, Davies WJ, Reynolds MP, Dodd IC (2016) Foliar abscisic acid-to-ethylene accumulation and response regulate shoot growth sensitivity to mild drought in wheat. Front Plant Sci 7:461. https://doi.org/10.3389/fpls.2016.00461

Varshney RK, Paulo MJ, Grando S, van Eeuwijk FA, Keizer LCP, Guo P, Ceccarelli S, Kilian A, Baumd M, Graner A (2012) Genome wide association analyses for drought tolerance related traits in barley (*Hordeum vulgare* L.). Field Crop Res 126:171–180. https://doi.org/10.1016/j.fcr.2011.10.008

Vavilov NR (1951) The origin, variation, immunity and breeding of cultivated plants. Chron Bot 13:1–366

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93(1):77–78. https://doi.org/10.1093/jhered/93.1.77

Voss-Fels K, Frisch M, Qian L, Kontowski S, Friedt W, Gottwald S, Snowdon RJ (2015) Subgenomic diversity patterns caused by directional selection in bread wheat gene pools. Plant Genom 8(2):plantgenome2015-03. https://doi.org/10.3835/plantgenome2015.03.0013

Warnes G, Gorjanc G, Leisch F, Man M (2013) Genetics. Population genetics, R package version 1.3.8.1. Available online at: http://CRAN.R-project.org/package=genetics

Wehner GG, Balko CC, Enders MM, Humbeck KK, Ordon FF (2015) Identification of genomic regions involved in tolerance to drought stress and drought stress induced leaf senescence in juvenile barley. BMC Plant Biol 15(1):125. https://doi.org/10.1186/s12870-015-0524-3

Wehner G, Balko C, Ordon F (2016a) Experimental design to determine drought stress response and early leaf senescence in barley (*Hordeum vulgare* L.). Bio-Protoc 6(5):e1749. https://doi.org/10.21769/BioProtoc.1749

Wehner G, Balko C, Ordon F (2016b) QTL for water use related traits in juvenile barley. Agronomy 6(4):62

Wiegmann M, Maurer A, Pham A, March TJ, Al-Abdallat A, Thomas WTB, Bull HJ, Shahid M, Eglinton J, Baum M, Flavell AJ, Tester M, Pillen K (2019) Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues. Sci Rep 9(1):6397. https://doi.org/10.1038/s41598-019-42673-1

Wójcik-Jagła M, Fiust A, Kościelniak J, Rapacz M (2018) Association mapping of drought tolerance-related traits in barley to complement a traditional biparental QTL mapping study. Theor Appl Genet 131(1):167–181. https://doi.org/10.1007/s00122-017-2994-1

Wosene GA, Berhane L, Bettina IGH, Karl JS (2015) Ethiopian barley landraces show higher yield stability and comparable yield to improved varieties in multi-environment field trials. J Plant Breed Crop Sci 7:275–291

Xiong J, Chen D, Chen Y, Wu D, Zhang G (2023) Genome-wide association mapping and transcriptomic analysis reveal key drought-responding genes in barley seedlings. Curr Plant Biol 33:100277. https://doi.org/10.1016/j.cpb.2023.100277

Yokota A, Takahara K, Akashi K (2006) Water stress. In: Madhava Rao KV, Raghavendra AS, Janardhan Reddy K (eds) Physiology and molecular biology of stress tolerance in plants. Springer, Dordrecht, pp 15–39

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14(6):415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zakhrabekova S, Gough SP, Braumann I, Müller AH, Lundqvist J, Ahmann K, Dockter C, Matyszczak I, Kurowska M, Druka A, Waugh R, Graner A, Stein N, Steuernagel B, Lundqvist U, Hansson M (2012) Induced mutations in circadian clock regulator *Mat-a* facilitated short-season adaptation and range extension in cultivated barley. Proc Natl Acad Sci 109(11):4326–4331. https://doi.org/10.1073/pnas.1113009109

Zhang X, Shabala S, Koutoulis A, Shabala L, Zhou M (2017) Meta-analysis of major QTL for abiotic stress tolerance in barley and implications for barley breeding. Planta 245(2):283–295. https://doi.org/10.1007/s00425-016-2605-4

Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, Tang C, Toomajian C, Zheng H, Dean C, Marjoram P, Nordborg M (2007) An Arabidopsis example of association mapping in structured samples. PLoS Genet 3(1):e4. https://doi.org/10.1371/journal.pgen.0030004

Zhao Y-F, Peng T, Sun H-Z, Teotia S, Wen H-L, Du Y-X, Zhang J, Li J-Z, Tang G-L, Xue H-W, Zhao Q-Z (2019) miR1432-OsACOT (Acyl-CoA thioesterase) module determines grain yield via enhancing grain filling rate in rice. Plant Biotechnol J 17(4):712–723. https://doi.org/10.1111/pbi.13009